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The Occurrence and Destructiveness of Clitocybe Root Rot of Woody Plants in Florida

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CONTENTS

INTRODUCTION

Extensive investigations have been made from 1924 to 1944 on the death of trees and other woody plants in Florida from mushroom root rot caused by *Clitocybe tabescens* (Fr.) Bres. The interest of the writer in this problem was engendered by his study (16) of this fungus as the cause of root rot of grapevines in Missouri and the comparison of his Missouri isolate with one from a Florida specimen of eucalyptus studied by Richards (41). Collections of this fungus in the herbarium of the Florida Agricultural Experiment Station and the frequency with which it was found growing around Gainesville and at other points when the

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writer came to Florida late in 1923 indicated that it was of rather common occurrence in this State. However, published records of the occurrence of Clitocybe root rot in Florida were found to be limited to brief reports by Hole (12) and Fawcett (9) on peach trees.

Late in 1924 and early in 1925 a number of guava (*Psidium guajava*) trees in the vicinity of Cocoa were found dying from root rot and *C. tabescens* was isolated. The subsequent finding of this disease attacking a great diversity of woody plants, including citrus, at other points in the State indicated that the disease was of considerable economic importance and work on it as an active project was begun in 1931. The records that the writer has had the opportunity of securing on its occurrence and distribution in the State have been supplemented by specimens that have been sent to the Florida Agricultural Experiment Station at Gainesville for diagnosis over a period of 25 years and collections by members of the Plant Pathology Department, to whom he desires to express his appreciation for their interest and helpful cooperation. Progress reports of the investigations on this disease in Florida have been published over a period of several years (17 to 40).

HISTORICAL

The earliest records of what undoubtedly was Clitocybe root rot in Florida are provided by inquiries of growers to the editors of the early Florida agricultural periodicals, in regard to the sudden dying of guava trees. These brief accounts are so descriptive of this disease, which has been observed repeatedly on guava and other woody plants since 1924, that they constitute convincing evidence that it occurred in Florida at least as early as 1885.

In this year, Bacon (3) of Ormond (north of Daytona Beach) made the following inquiry:

> While you are on the guava question, will you or some of your readers inform us of the sudden and premature death of many of the common kind of guava trees; I am informed the Cattley and Chinese are affected the same way. The tree will appear to be in a perfectly healthy condition, the next day the leaves will begin to wither and drop off. The bark and wood of the tree above ground will appear sound and perfectly healthy; while the roots at the same time will appear to have been in a state of decay for some time—the bark soft and slipping easily from the wood. . . .

In 1889, E. W. A. (2) of the same locality inquired as follows:

> Have you or any of your subscribers had guavas or Medlars die of what seems to be a disease of the roots? The outer bark of the roots turns black, also the main trunk for 2 or 3 inches above the ground. The first indications of the disease are the leaves falling. It is neither root-knot nor girdlers. I have lost two Medlars and five or six large guava bushes recently, and on inquiry of my neighbors I find that they have been more unfortunate than I. Can any one name the disease and give a remedy?

The editor stated that he was of the opinion that this was a variety of foot rot and that it probably could be cured by removing all the diseased bark from the trunk and roots and painting with coal tar. He also stated that there undoubtedly was a fungus growth involved and that he would like to hear from others on the same subject.

In 1890, Carson (4) of Midland[1], Polk County, made the following inquiry:

> Can you ar any of your readers tell what quality of land suits guava growth best? I am greatly discouraged with mine on sand hill land where clay is near the surface. In one spot in my guava grove, just where there is a cluster of willow oaks, and a grapevine to every bush and tree (I mean an average) nearly all my plants died the third year. They die both with and without fruit. Some died at one year old, after a remarkable growth. I can see nothing in the shape of insects to kill them. They go, root and branch, first the tips and then the main boughs, then the body and last the roots.

Later in the year Carson (5) related that he dug up some trees when dying, and some soon after death, and found nothing but a kind of dry rot which appeared to begin at the extremities of the roots. He stated that he had lost at least 10 percent of his planting and that trees were still dying. He added that in one area of about 80 square feet, where there was a dense willow oak thicket with pine trees and grapevines, every guava bush died. His description indicates that he planted his guavas on uncleared land.

The following reply was written by Mitchell (13) of Daytona (now Daytona Beach):

> In answer to S. W. Carson, Midland, Polk County, we have had no end of the trouble he reports with guava bushes.
>
> My neighbor, C. P. Land, after careful investigation, is convinced it is caused by the smothering of the root crown—right where the roots start—by sand and bark long enough to develop a fungoid growth on the crown, and that keeping the crown clean will prevent this growth, which, beginning at the bark, eats its way through the sap and then the bush dies. The roots sometimes die when the circulation of the sap is cut off, but more often they throw up shoots below the crown, which can be thinned out to replace the original body.
>
> If bushes with half grown fruit, when they are not yet eaten clear around, are cleaned off and the fungoid part scraped off, the bark will heal, the fungoid growth cease, and probably the fruit will ripen, if not too late.

The earliest authentic record, which did not come to the writer's attention until 1931, is based on the observations of Dr. H. Harold Hume. In the files of the Florida Agricultural Experiment Station are photographs, made by Hume on October 13, 1902, illustrating the occurrence of *C. parasitica*, as the fungus was then designated, on a peach tree at his residence in Lake City, where the Station was then located. This record was made about a year and a half after Wilcox (44) described this fungus as the cause of a root rot of fruit trees in Oklahoma. Hume later turned over to the writer notes he had made, giving a description of the fungus and the symptoms of the attacked peach tree. He recorded finding this fungus attacking 3 trees of *Prunus persica*, 4 of *Prunus caroliniana*, 7 or 8 of *Quercus laurifolia*, 1 of *Carya olivaeforme* [*C. illinoënsis*], and 1 of arborvitae or *Juniperus* sp.

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[1]While Carson wrote from what was then Midland, his home place was on the east shore of Lake Clinch and now in the city limits of Frostproof. In this connection it is of interest to mention that the writer in recent years has observed a number of instances of the dying of guava and various other woody plants from Clitocybe root rot in properties in this immediate vicinity and also at other points in the central part of Polk County. It is thus apparent that the disease that was so troublesome to Carson still continues to cause losses of guava trees.

In a brief discussion of peach diseases in Florida in 1905, Hole (12) stated that root rot, caused by *C. parasitica*, was troublesome in orchards on newly cleared, moderately high hammock land at Fulton, east of Jacksonville, on the lower St. Johns River.[2] He described the symptoms of trees attacked by this fungus and stated that, upon applying to the Experiment Station for assistance, Professor Hume came to investigate and diagnose the trouble.

Fawcett (9), in 1911, mentioned Clitocybe root rot as one of the peach diseases being studied by O. F. Burger. The symptoms of this disease were described in an unpublished manuscript on peach diseases in Florida by Burger, dated 1911, found in the files of the Experiment Station in 1931. Mention was made of the fungus fruiting at the base of an 18-year-old peach tree that was dying, and a photograph also was found in the files. He also found the fungus fruiting on the broken root of a chinaberry (*Melia azedarach*) tree in the same yard at Dade City where this peach tree occurred and stated that another peach tree affected by this disease had been found on the Experiment Station grounds at Gainesville.

In March, 1920, H. E. Stevens, then plant pathologist of the Florida Agricultural Experiment Station, sent to the Forest Products Laboratory at Madison, Wisc., for identification of the causal organism, a specimen of a eucalyptus tree that had died from root rot. Cultures made from this by Siggers yielded a rhizomorph-producing fungus which was thought to be *Armillaria mellea* but, after developing a cluster of sporophores, was identified by C. J. Humphrey as *C. monadelpha* (=*C. tabescens*).[3] The comparative study of the cultural characteristics of this fungus with those of the closely related root-rot fungus, *A. mellea*, begun by Siggers prior to his leaving for Central America, was continued by Richards (41).

ECONOMIC IMPORTANCE

While *C. tabescens* has been reported to occur in the United States as far north as New York and Michigan, and as far west as Kansas, root rot caused by this fungus is known to occur only in the southeastern States, where it has been reported from eastern Texas, Oklahoma, Arkansas, Missouri, southern Illinois and Indiana, West Virginia and Maryland southward to Florida and the inland Gulf States. The extensive investigations of the writer, conducted more or less actively from 1924 to 1944, and intermittently since then, have shown that this disease is of widespread occurrence, and often highly destructive, throughout a large part of Florida, where, in addition to native forest trees, it attacks tung-oil trees and a large variety of fruit and ornamental trees, shrubs and vines, including numerous exotic plants. Losses on numerous properties examined vary from an occasional ornamental tree or shrub to serious losses of the same continuing over a period of several years, the loss of scattered trees or groups of trees in commercial plantings of citrus, tung-oil and peach, or the loss of a majority of the trees in

[2]These orchards were abandoned many years ago and the land has reverted to hammock forest.

[3]Siggers, Paul V. Summary of comparative study between forms of Armillaria and Clitocybe. Typewritten report prepared in 1921.

windbreak and roadside plantings of the highly susceptible casuarina trees (*Casuarina* spp.).

The economic importance of Clitocybe root rot varies greatly according to the prevalence of the fungus in local areas, the age and extent of the planting, its esthetic or commercial value, and the susceptibility of the plant species to the disease. On occasions when the writer has been called upon to diagnose the cause of rapid decline or death of some tree or shrub that proved to be due to Clitocybe root rot a careful inspection of the premises resulted in finding one or more additional specimens attacked, which the owner did not even suspect of being unhealthy. In many instances the owner recalled additional specimens having died under similar circumstances. The occasional loss of a plant in a yard rarely causes the owner much concern unless it was a particularly prized specimen. However, in properties where losses of plants occur repeatedly over a period of years the high mortality may be a cause of considerable concern. When it is considered that such losses occur on a large number of properties about the State, it is evident that the aggregate losses in ornamental plantings alone become of considerable importance.

During the course of the writer's investigations on Clitocybe root rot in Florida from 1924 to 1944 certain properties in various parts of the State were found to be veritable hotbeds of infection for the fungus, with trees and shrubs dying at intervals for years. Properties where this root-rot fungus has proved unusually destructive invariably have been hammock, pine-oak or scrub types of land on which various species of oak trees abounded prior to clearing, the infected roots of standing trees and stumps, or those left in the ground at the time of clearing, serving as centers of infection to plantings made subsequently. Outstanding examples of such severely infected properties have been found near Brooksville and Blanton on the West Coast, at Babson Park, Lake Alfred, Oakland and Fruitland Park in the central part of the State, and at Artesia and points on Merritt Island on the East Coast. In one property at Lake Alfred the repeated extensive losses from root rot, together with periodic cold injury, have proved so exasperating that the owner, whose hobby is the non-commercial growing of exotic plants, decided to sell his place and move to a more favorable locality. Periodic inspection of the plantings occasionally developed on such heavily infected properties, aided by the cooperation of the owners, has enabled the writer to record many new or unusual exotic plants as hosts for the Clitocybe root-rot fungus.

The plant failures from root rot just described often represent a distinct financial loss in addition to loss of time and commercial or esthetic value. In one estate where the owner had specialized in large specimens of arborvitae and various horticultural forms thereof, at an initial cost of $5.00 per tree, 16 trees were found either dead or dying after 3 years of care.

One of the writer's former colleagues in the Florida Agricultural Experiment Station, Dr. W. B. Shippy, who made an extensive study of rose diseases while stationed at Leesburg, concluded that 75 percent of the mortality of rose bushes investigated by him in Florida was due to Clitocybe root rot.

In sections of Florida where the highly susceptible casuarina trees are used as hedge, ornamental, screen, windbreak or roadside plantings the inroads of the Clitocybe root-rot fungus within a few years frequently results in such widespread mortality that their usefulness or esthetic value is often soon materially diminished or even virtually completely destroyed. In a few instances hedge of the horsetail casuarina (*C. equisetifolia*) that have been rendered unsightly by extensive losses from root rot have been removed and replaced by some other kind of plant. The high mortality in plantings of casuarina trees in Florida due to root rot has been described in detail by the writer (38).

Commerical peach culture has been tried periodically in Florida since about 1885 but the plantings invariably have proved short-lived. It was pointed out many years ago by Hole (12) that Clitocybe root rot was quite destructive to peach trees but this fact appears to have been largely overlooked since the majority of the blame for the repeated failures has been attributed to root knot, caused by nematodes. That root rot is still highly destructive to peach trees is attested by the readiness with which both budded and seedling trees are attacked in yard plantings in various parts of the State.

In 1940, Thornton (42), in giving the results of his experience with a 20-acre planting of 1400 Jewell trees on peach stock near Blanton, Pasco County, stated that Clitocybe root rot had made such extensive inroads into this planting that by the time the trees had attained an age of 3½ years 192 had died, 146 were so seriously affected that they would probably die within a year, and that about 200 others were known to be slightly attacked. According to these figures, this disease had attacked a total of 38 percent of the trees at a very early age. After inspecting this planting, the writer also found a considerable number of trees to be dead or dying from Clitocybe root rot in a 3-year-old peach orchard on another property in this section. Both these plantings were on high, rolling pine-oak land, on which numerous centers of infection might be expected to exist in the soil. The above evidence indicates that this disease must have been an important factor, in addition to the much publicized root knot, in the failure of earlier peach plantings in Florida. In 1952 the writer[4] found that Clitocybe root rot also was widespread and destructive in Georgia and South Carolina peach orchards, where the disease had not been reported previously.

Clitocybe root rot also has been found to occur more or less frequently in commercial plantings of tung-oil trees (31) and in citrus groves (37) on rough lemon stock in various parts of Florida and has appeared in recent plantings of lychee.

SYMPTOMS

The symptoms of Clitocybe root rot vary considerably with the kind and size of the plants attacked and the rapidity with which girdling occurs. Root rot symptoms usually do not become apparent until the fungus has invaded the root system or root crown,

[4]Rhoads, Arthur S. Clitocybe root rot found widespread and destructive in Georgia and South Carolina peach orchards. Plant Dis. Reptr. 38(1): 42–46. 1954.

or both, sufficiently to interfere with the life processes of the host plant. The dying of small trees or shrubs may follow rapidly, while that of large ones proceeds much more slowly, with symptoms of decline showing for sometimes a year or two before death.

FIG. 1. Portion of 10-year-old scalybark casuarina (*Casuarina lepidophloia*) windbreak, showing tree nearly completely defoliated from girdling by Clitocybe root rot in contrast with healthy trees on either side.

On conifers, such as arborvitae and pines, a slight yellowing of the foliage may occur occasionally but, as a rule, at least on small trees, the foliage on the first limbs to become affected by the progress of the invading fungus simply turns brown and dies and the decline of the tree progresses more or less rapidly.

Small broadleaf trees and shrubs frequently are killed so rapidly that they may not develop any particular symptoms until they begin to wither and die. Larger trees and shrubs usually show more or less yellowing and defoliation, accompanied by the rapid wilting and dying of individual limbs or trunk divisions. Finally the whole plant dies. In tung-oil trees the leaves developing the year following partial girdling tend to be reduced in size, sparse and chlorotic, becoming progressively so towards the tops of the trees, and later wilting more or less rapidly. There is also a marked reduction in the size of the nuts developing. The wilting frequently has its inception on the limbs or trunk divisions on one side of a tree or may develop more or less simultaneously over the entire crown. Peaches, plums, guavas and loquats may develop a thin, sickly appearance of the foliage, with dying of some branches, or actively growing trees may shrivel and die rapidly before the fruit matures, in which case it mummifies on the trees.

In the case of the casuarinas the first symptom usually is a slight yellowing of the foliage branches[5], which generally develops first on the lower limbs of the crown on the side where the roots are first attacked. This yellowing soon becomes more conspicuous and general and is accompanied by a pronounced shedding of the affected foliage branches. As the disease progresses the crown acquires a thin, sickly appearance and an unusually heavy litter of shed foliage branches accumulates under the tree. Attacked trees generally become about three-fourths defoliated, and often completely so on the terminal portions of the crowns, before dying (Fig. 1). However, in very young trees where girdling proceeds quite rapidly the foliage branches over the entire crown may turn brown and shrivel within a brief period without any pronounced yellowing or shedding. In older trees, where girdling may require 2 or 3 years from the time the first symptoms become apparent, new foliage branches may develop following the shedding of a large portion, but the new ones usually attain only about half the normal length for the species. The symptoms of this disease on casuarinas have been described in detail in a separate paper (38).

Aside from the size of the plants, the rapidity of girdling and death depends upon the rate of growth and spread of the fungus as determined by soil moisture and temperature conditions. When the fungus develops rapidly during warm, rainy periods and girdling is completed in a short space of time, wilting and dying usually progress quite rapidly. However, plants extensively girdled by root rot usually appear to die most rapidly during periods of drought. This obviously is due to the added demands for water made upon the roots at such times.

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[5]In these trees the leaves are reduced to whorls of scale-like teeth at the nodes of the dehiscent foliage branches, which may appear to the layman as needle-like leaves.

By the time a pronounced yellowing and defoliation or other decline of the tops of attacked trees becomes apparent, the mycelial sheets of the fungus usually have progressed upward on the bases of the trunks so that their presence can be verified by making a cut in the bark down to the wood. In thin-barked trees, merely slicing away a bit of the outer bark frequently will suffice to disclose it. The upward progress of the mycelium at the base of the attacked tree may be accompanied by a slightly sunken lesion which results from failure of the invaded tissues to keep pace in growth with those of the rest of the trunk. Such basal lesions are most conspicuous on attacked trees and shrubs with thin bark, such as guava and Surinam-cherry, and do not develop on thick-barked ones such as eucalypts and pines. They frequently develop first on one side before encircling the trunk. By the time the crown begins to become thin from defoliation and dying

FIG. 2. Base of large guava (*Psidium guajava*) tree killed by Clitocybe root rot, showing lesions extending up trunk divisions with dead bark cracked loose on one at right and sloughed off on two at left.

of branches, the cutting test of the bark usually shows the base of the tree immediately above the ground to be completely girdled. This girdling is often accompanied by longitudinal cracking of the bark as it dies, and sometimes also by a pronounced cracking, demarking the marginal limit of the lesion (Fig. 2). The basal lesions usually extend upward from 4–6 inches to about a foot, and rarely as high as 2 feet, above the ground before the attacked tree dies, the height frequently being greater on one side than on the other. It varies according to the size of the attacked tree and the rapidity of death and usually is greater on large trees than on small ones. The mycelial

sheets that develop under the bark are coextensive with the lesions (Fig. 3). In the casuarinas the basal girdling by root rot has been shown (38) to stimulate a pronounced hypertrophy of the trunks above the girdled portions, which is a characteristic feature of the disease in certain species but not developing in others. Stone-fruit trees, such as peach, plum and Carolina laurelcherry, commonly develop more or less gum formation in the cambial region, which may be so copious as to exude through cracks in the bark. Citrus trees attacked by root rot also show exudation of gum occasionally. The symptoms of this disease on the latter have been described in detail in a separate paper (37).

FIG. 3. Large guava (*Psidium guajava*) tree dying from Clitocybe root rot, with dead bark cut away on basal lesion to show extent to which the mycelium has spread up and around trunk.

When the bark of roots attacked by Clitocybe root rot is peeled off the mycelium of the fungus is seen to have developed between the bark and the wood (Fig. 4) and also through the inner layers of the bark. This varies from thin, filmy wefts to sheets that are white when freshly developed but soon become cream- to chamois-colored with age. The mycelial sheets often show a more or less radiating, fan-shaped type of growth (Fig. 4), but this feature is less conspicuous than usually occurs with *Armillaria mellea* and is less apparent in old

FIG. 4. Roots of Bourbon Dombeya (*Dombeya punctata*) bush dying from Clitocybe root rot, with bark peeled off, showing luxuriant growth of mycelial sheets developed during hot, rainy weather. Note thick waxy margin and perforate character of older portions.

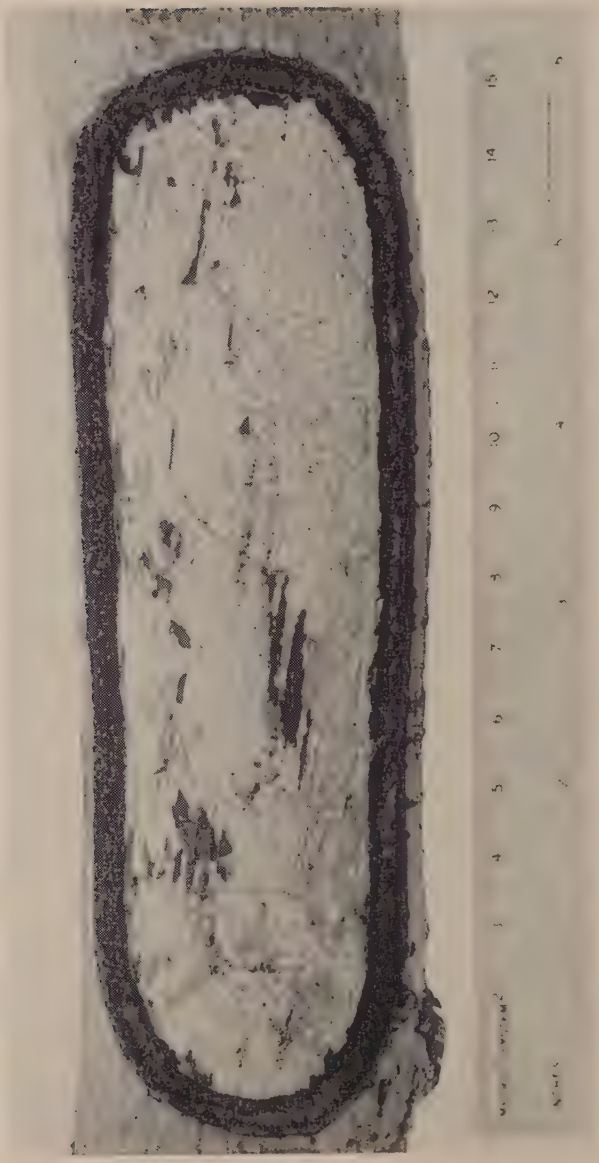

FIG. 5. Root of scrub hickory (*Carya floridana*) attacked by *Clitocybe tabescens*, with area of bark removed, showing perforate character of the mycelial sheet. From stump left in land cleared 5 years previously.

compact mycelial sheets. When especially favorable soil moisture conditions stimulate an unusually luxuriant growth, the advancing margins of the mycelial sheets may be considerably thickened (Fig. 4). In addition to mycelial sheets of considerable extent, the fungus also commonly develops flattened, narrow to broad, thallus-like structures of the same color and either entire in outline or with numerous thread-like lateral branching from the margins. These structures, which are essentially rhizomorphic in character, also develop more or less abundantly in pure cultures of the fungus. Freshly dug roots in which the mycelium is still growing are characterized by a pronounced mushroom or fungus odor.

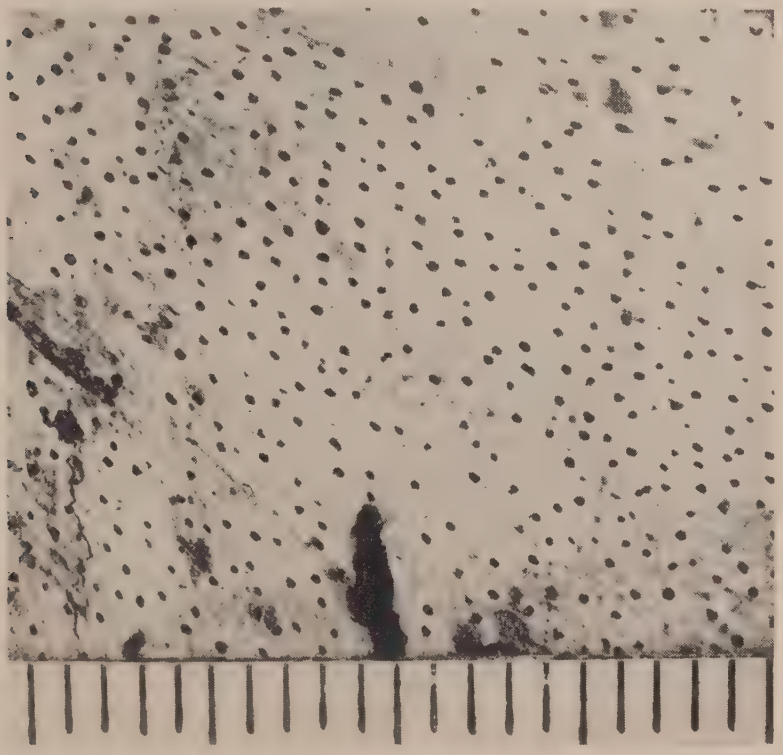

FIG. 6. Portion of mycelial sheet developed between bark and wood of root of sand pine (*Pinus clausa*) killed by Clitocybe root rot, showing perforate character and suggestion of radial growth. Millimeter scale shown, enlarged 5 times.

An additional feature of the mycelial sheets is the frequent occurrence of a peculiar perforate character clearly apparent even to the unaided eye (Fig. 5). These perforations, which occur at frequent but varying intervals, are rounded or slightly elongated radially and sometimes quite minute but average about 0.5 mm. in diameter, with the large ones attaining a diameter of 1 mm. (Fig. 6). In examining a series of freshly dug roots of a number of plants attacked by Clitocybe

root rot it was noticed in peeling off pieces of bark the mycelial sheets usually adhered to the wood but sometimes adhered to the bark, or they may split with part adhering to each. In examining the perforate-appearing places with a hand lens they are seen to consist of islands of whitish, watery tissue studding the mycelial sheets at these points. These islands of watery tissue frequently remain attached to the inner surface of the bark or the outer surface of the wood and in such cases leave a corresponding series of holes in the mycelial sheet. When a group of these structures was viewed sidewise they appeared as a series of papillae or short, rounded to elliptical columns which were watery white and glistening. Upon maceration on a slide, microscopic examination revealed them to consist of thin-walled parenchymatous tissue, obviously developed in some way from the host plant. While their mode of origin is not understood, it is apparent that there is no correlation between frequency of occurrence and the much more

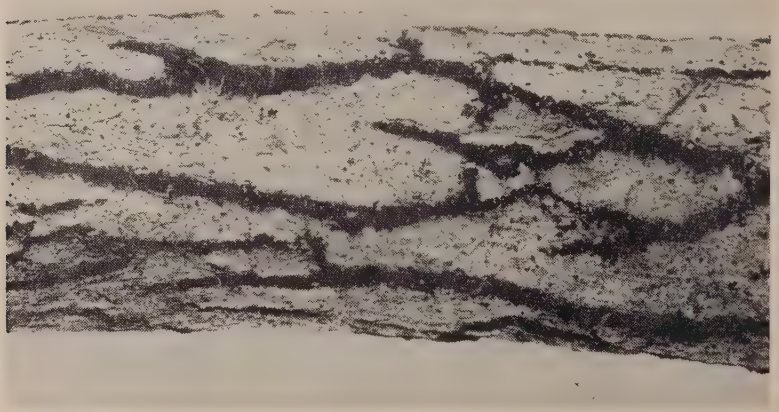

FIG. 7. Root of seedling sweet orange (*Citrus sinensis*) tree attacked by Clitocybe root rot, showing blackish xylostroma extrusions developed through longitudinal fissures in the bark. Natural size.

numerous medullary rays. The details of this perforate character of the mycelial sheets resulting from the formation of these islands of parenchymatous tissue are best observed in young, whitish mycelial sheets. In older sheets that have become chamois-colored these structures become sodden and discolored. This perforate effect of the mycelial sheets has been observed with great frequency in roots of a diverse array of plants attacked by Clitocybe root rot, and even in a rhizomorphic sheet of the fungus developed between the concentric leaf bases of a banana stem (29). It persists in dried specimens and appears to be a character of diagnostic value for *C. tabescens* and one that apparently is not found in *A. mellea*.

The black, rounded or flattened, cortical or hypogeal shoestring-like rhizomorphs so frequently accompanying the well-known honey agaric, *A. mellea*, have not been observed to occur in the case of *C. tabescens*. However, a peculiar development of blackish, indurated, sometimes

frilly xylostroma extrusions, resembling the stroma of certain Pyrenomycetes (Fig. 7), is frequently found on the bark of roots attacked by Clitocybe root rot. These formations do not appear at all in many cases but develop abundantly in others. They develop on both large and small roots but are larger and more prominent on the former, where they attain a width of as much as $\frac{3}{16}$ of an inch and a length of several inches. The development of these structures also has been reported by a number of investigators in the case of the root rot caused by the closely related root-rot fungus, *A. mellea*, and some have regarded them as rhizomorphs. Their origin and anatomy have

FIG. 8. *Clitocybe tabescens* fruiting at base of common guava (*Psidium guajava*) attacked by root rot.

been discussed in detail by the writer (37), who has shown that they are simply outgrowths of the mycelial sheets developed between the bark and the wood, and which become extruded through longitudinal fissures formed in the bark as a result of growth pressure.

The wood of the larger attacked roots often develops both radial and concentric cracks which become filled with whitish sheets of mycelium. In several instances, particularly in citrus trees, this invasion of the wood has been found to develop even into the butt of the tree. In well-advanced cases of the disease the fungus causes a soft, whitish, delignifying type of decay of the deeper-lying roots and the decayed wood is usually watersoaked and soft and spongy, usually becoming distinctly gelatinous in the late stages of decay.

An additional symptom of Clitocybe root rot is the frequent occurrence of one or more clusters of the mushroom fruiting bodies of the fungus at the bases of trees or shrubs in which the disease has been

developing for some time (Fig. 8). In fact, these often develop long before any other symptom of the disease becomes apparent, and simply finding a cluster of the fruiting bodies at the base of a tree, particularly on citrus trees (37), has rendered it possible to locate and treat a

FIG. 9. A, Cluster of mushrooms of *Clitocybe tabescens* that developed on oak root left in cleared land, showing caespitose character. B, Under side of the same cluster of mushrooms shown in A, illustrating decurrent gills and lack of an annulus.

surprisingly large number of attacked trees before the presence of the disease was even suspected. The experience of the writer has shown that the development of fruiting bodies of *C. tabescens* at the base of a living tree is an infallible sign that this fungus has already invaded the root system more or less extensively.

The production of fruiting bodies by this fungus depends largely upon the progress of the disease, the seasonal conditions, and the size of the host plant. In Florida, they develop with greatest frequency during the fall and early winter, from about the middle of September to the middle of December, but may develop also in other months if favorable soil moisture conditions prevail. The clusters usually develop from the root crown but occasionally may develop a short distance away on one of the lateral roots. Usually only one or two clusters develop during the year, and in many cases none develop. As a rule, the larger the tree, the more clusters the fungus develops; small shrubs often produce none.

CAUSAL FUNGUS

Clitocybe root rot is caused by *Clitocybe tabescens* (Fr.) Bres., one of the gill fungi. The fruiting bodies of this fungus usually consist of a few to many individuals, with the stems developing from a common base[6] (Fig. 9, A and B). When fully developed, the caps are convex to flattened or centrally depressed with age, whitish to light tan or honey-colored, smooth or adorned with tufts of fibrils near the center, and from 2–3½ inches in diameter, with whitish gills underneath (Fig. 9B). The united Clitocybe, as this fungus is commonly called, resembles the closely related honey agaric (*Armillaria mellea*) in habit of growth, color, texture, and general appearance but is distinguished principally by the absence of an annulus on the stem, dissimilar spores, and in being more slender from the beginning. It is considered superior to *A. mellea* in flavor and edibility. The latter fungus is of cosmopolitan occurrence and causes a very similar root rot. A comparative study of these two closely related root-rot fungi have been made the basis of a special paper (35). *A. mellea* occurs more or less frequently in hammock forests of northern Florida but in this State it is largely supplanted by *C. tabescens*. The mushrooms of the latter fungus usually attain their full development within a few days from the time the young "buttons" appear and decay rapidly in warm, rainy weather. However, if dry weather follows their development the clusters may dry up and turn dark brown to blackish but remain recognizable for several weeks to one familiar with the fungus. At maturity, however, the clusters frequently deliquesce or rot down into a thin, dark brown, circular layer on the ground, which may remain apparent for some time.

[6] In one of three compact clusters of fruiting bodies that were beginning to develop at the base of a large laurel oak a count of the miniature sporophores revealed a total of 381, the majority having caps averaging about ⅛ inch in diameter. However, while excessively large numbers of sporophores usually begin to develop in the monadelphous clusters, as a rule only a few of the faster growing ones develop to maturity, the rest becoming aborted.

DISTRIBUTION OF THE DISEASE

The available data accumulated from 1924 to date show that Clitocybe root rot has been found in Florida from Fernandina, in the northeastern corner, west to DeFuniak Springs in western Florida, and south to Lake Okeechobee in the interior of the peninsula, Fort Lauderdale and Davie on the lower East Coast, and Naples on the lower West Coast. No authentic records of the occurrence of this disease

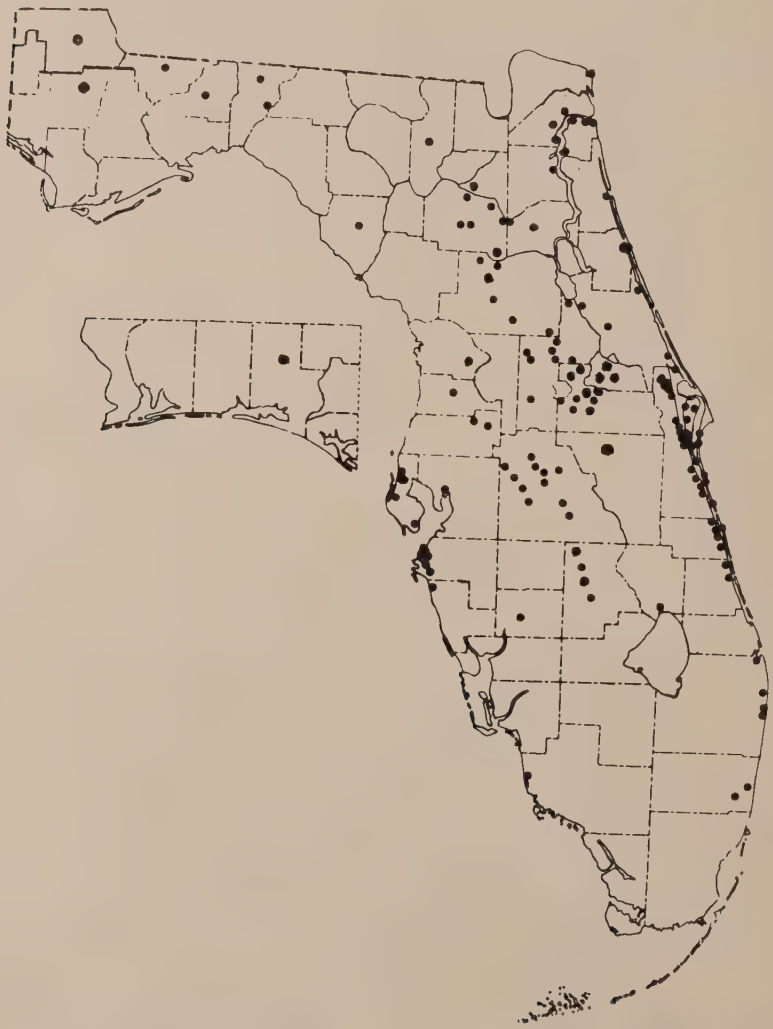

FIG. 10. Recorded distribution of Clitocybe root rot in Florida, the dots indicating localities where the disease has been found on from one to more than 50 plants.

have been secured west of DeFuniak Springs, though there is ample reason to believe that it occurs there. In peninsular Florida the disease is of particularly frequent occurrence along the middle East Coast and through the central portion and part of the less developed West Coast (Fig. 10).

The writer's opportunities for observing the occurrence of Clitocybe root rot in Florida were limited largely to the citrus region for many years but brief visits to the northern part of the State have since been made. Extensive areas of the State still remain undeveloped so far as plantings, other than farm crops, are concerned, and many areas, particularly in the extreme southern part, remain inaccessible. Further inspection of plantings throughout the State unquestionably will reveal still greater distribution of the disease and additional hosts. Many instances of its occurrence have never been recorded because growers and nurserymen frequently remove and destroy plants as they are found dying.

RECORD OF HOST PLANTS

A special effort has been made to keep a record of the number of times plants of different kinds have been attacked by Clitocybe root rot. Aside from the general interest afforded by such a host list, the frequency with which the occurrence of the disease has been noted on certain widely planted species affords an indication of their relative susceptibility. This list has been limited to instances where the disease obviously was responsible for the death of plants and does not include its saprophytic occurrence. Where various kinds of trees were budded on some other kind used as a rootstock, the hosts are listed under the rootstock, so far as this was known. For example, where several different kinds of citrus trees were all on rough lemon stock the latter is considered the host rather than the particular kind of tree budded thereon. This host list, as thus given in Table 1, comprises 213 species of plants belonging to 137 genera and 59 families.[7] Of these, the Casuarinaceae. Rosaceae, Leguminosae, Rutaceae, and Myrtaceae are represented with outstanding frequency. Conifers are attacked as well as broadleaf or hardwood trees, and even bananas and palms have been found attacked. To avoid repetition, records of isolations of the causal fungus are listed on the right side of this table, giving the year, condition and locality of the host, and whether or not the fungus fruited in culture.

[7]The usage of scientific and common names conforms largely to that of the following authoritative source: Kelsey, Harlan P., and William A. Dayton. Standardized plant names: a revised and enlarged listing of approved scientific and common names of plants and plant products in American commerce or use. 2nd edition. J. Horace McFarland Co.: Harrisburg, Pa. 1942. Departures in usage occur, however, where subsequent changes in nomenclature have been made. The Check list of the native and naturalized trees of the United States, including Alaska, published in mimeographed form by the U. S. Forest Service in 1944, also has been consulted.

TABLE 1.—*Records of the host plants for Clitocybe tabescens and isolations of the fungus in Florida.*

Records of occurence			Records of isolations				
Scientific name	Common name	No. attacked	Year cultured	Condition of host	Locality		Fruited in culture
					Postoffice	County	
GYMNOSPERMAE							
PINACEAE							
Cedrus deodara (Roxb.) Loud.	Deador Cedar	3	1943	Dying tree	Quincy	Gadsden	Yes
Pinus elliottii Engelm. var. *densa* Little & Dorman	South Florida Slash Pine	2	1931	Dead tree	Bonaventure	Brevard	No
			1938	Stump in cut-over land	Merritt, Merritt Island	Brevard	Yes
Pinus clausa (Engelm.) Vasey	Sand Pine	13	1932	Dead tree	Grand Island	Lake	No
			1932	Dead tree	Cocoa	Brevard	Yes
			1933	Dead tree	Cocoa	Brevard	No
			1934	Dead tree	Cocoa	Brevard	Yes
Pinus echinata Mill.	Shortleaf Pine	7					
Pinus glabra Walt.	Spruce Pine	1	1944	Dying tree	Gainesville	Alachua	Yes
Pinus palustris Mill.	Longleaf Pine	3	1937	Declining tree	Brooksville	Hernando	Yes
Pinus taeda L.	Loblolly Pine	3					
CUPRESSACEAE							
Callitris glauca R. Br.	Blue Cypress-pine	21[1]					
Chamaecyparis lawsoniana (A. Murr.) Parl.	Port Orford Cedar	1					
Cupressus arizonica Greene	Arizona Cypress	2	1933	Dead treee	Bonaventure	Brevard	Yes
Cupressus sempervirens L.	Italian Cypress	4	1937	Dead tree	Lake Alfred	Polk	Yes
Cupressus sempervirens, var. *stricta* Ait.	Pyramidal Italian Cypress	1					
Cupressus sp.	Cypress	1					
Juniperus chinensis L., Cl. Pfitzer (*pfitzeriana* Spaeth)	Pfitzer Juniper	5					
Juniperus silicicola (Small) Bailey	Southern Redcedar	1					
Thuja occidentalis L.	American Arborvitae	36	1931	Dead tree	Cocoa	Brevard	Yes
			1932	Dead tree[2]	Gainesville	Alachua	Yes
			1932	Dead tree	Cocoa	Brevard	Yes
			1937	Dead tree[2]	Quincy	Gadsden	Yes

Cl. Heath (*ericoides*: *Retinispora ericoides* Hort.)		1					
Thuja orientalis L. (including various unidentified horticultural forms)	Oriental Arborvitae	15					
Cl. Berckmanns (*aurea nana*)	Berckmanns Arborvitae	1					
Cl. Rosedale Blue	Rosedale Arborvitae	2					
PODOCARPACEAE							
Podocarpus macrophyllus (Thunb.) G. Don, var. *maki* Endl	Shrubby Yew Podocarpus	2					
ANGIOSPERMAE							
MONOCOTYLEDONAE							
PALMACEAE							
Butia capitata Becc	Brazilian Butiapalm	3					
Livistona chinensis R. Br	Chinese Fanpalm	1					
Phoenix canariensis Chabaud	Canary Date Palm	3					
Phoenix dactylifera L	Date	1					
Serenoa repens (Bartr.) Small	Saw Palmetto	2					
ARACEAE							
Anthurium hookeri Kunth	Hooker Anthurium	1					
MUSACEAE							
Musa nana Lour	Dwarf Banana	1					
Musa paradisiaca L	Plantain (Orinoco or Horse) Banana	5					
Musa paradisiaca, var. *sapientum champa* Baker	Ladyfinger Banana	4	1931	Dead plant	Artesia	Brevard	No
			1931	Dead sucker	Artesia	Brevard	Yes
Musa sp	Banana	2	1931	Dead plant	Artesia	Brevard	Yes
			1931	Dead plant	Artesia	Brevard	Yes

[1]Represents only a few trees examined out of a large number lost in a planting in woods at Chinsegut Hill Sanctuary near Brooksville. About 20 additional trees also were lost in a planting near Fruitland Park. The total number is estimated at 100.

[2]Isolated by Mr. Erdman West.

TABLE 1.—(Continued)

Records of occurrence			Records of isolations				
Scientific name	Common name	No. attacked	Year cultured	Condition of host	Locality		Fruited in culture
					Postoffice	County	
DICOTYLEDONAE							
CASUARINACEAE							
Casuarina cunninghamiana Miq.	Cunningham Casuarina	165	1935	Dead tree	Audubon, Merritt Island	Brevard	Yes
			1937	Dead tree	Brooksville	Hernando	Yes
Casuarina equisetifolia L.	Horsetail Casuarina	1544[3]	1932	Dead tree	Georgiana, Merritt Island	Brevard	Yes
			1940	6 dead trees (4 inoculated)	Cocoa	Brevard	Yes
Casuarina glauca Sieb.	Swamp Casuarina	22	1940	2 dying trees	Cocoa	Brevard	No
Casuarina lepidophloia F. v. M.	Scalybark Casuarina	660	1931	Dying tree	Cocoa	Brevard	No
			1932	Dead tree	Cocoa	Brevard	No
			1932	Dead tree	Cocoa	Brevard	Yes
			1935	Dead tree	Cocoa	Brevard	Yes
			1939	Living tree (inoculated)	Lake Alfred	Polk	Yes
			1942	Living tree	Cocoa	Brevard	Yes
Casuarina montana Leschen.	Casuarina	16	1936	Dead tree	Courtenay, Merritt Island	Brevard	Yes
Casuarina stricta Dry.	Coast Casuarina	86	1936	Dead tree	Courtenay, Merritt Island	Brevard	Yes
			1937	Dead tree	Cocoa	Brevard	Yes
Casuarina spp.	Casuarina	20					
MYRICACEAE							
Myrica cerifera L.	Southern Waxmyrtle	1	1938	Dead tree	Merritt, Merritt Island	Brevard	Yes

JUGLANDACEAE							
Carya floridana Sarg.	Scrub Hickory	2	1932	Rootinground	Cocoa	Brevard	Yes
			1941	Dying tree	Cocoa	Brevard	Yes
Carya illinoënsis (Wangenh.) K. Koch	Pecan	2					
BETULACEAE							
Ostrya virginiana (Mill.) K. Koch	Eastern Hophornbeam	2	1944	Living tree	Gainesville	Alachua	Yes
FAGACEAE							
Quercus falcata Michx	Southern Red Oak	1					
Quercus incana Bartr	Bluejack Oak	8					
Quercus laevis Walt	Turkey Oak	19					
Quercus laurifolia Michx	Laurel Oak	15	1934	Dying tree	City Point	Brevard	Yes
			1941	Uprooted living tree	Mayport	Duval	Yes
Quercus myrtifolia Willd	Myrtle Oak	5	1937	Dead tree	Cocoa	Brevard	No
Quercus suber L.	Cork Oak	1					
Quercus virginiana Mill	Live Oak	1					
Quercus virginiana, var. *maritima* (Michx.) Sarg	Sand Live Oak	1					
Quercus spp	Oak	5	1937	Dead tree	Georgiana, Merritt Island	Brevard	No
MORACEAE							
Cecropia palmata Willd	Silverleaf Pumpwood	14	1933	Dead tree	Babson Park	Polk	Yes
Ficus benjamina L.	Benjamin Fig	1					
Ficus elastica Roxb	India rubber Fig	1[4]	1934	Living tree	Winter Haven	Polk	Yes
Ficus elastica, var. *variegata* Hort	Variegated India-rubber Fig	[4]					

[3]In addition to these it is estimated that more than 2,000 trees and 400 out of 1,000 replants died in windbreak plantings of one citrus grove between the Indian River and the Atlantic Ocean opposite Grant, and that several hundred others have died in extensive roadside plantings north of Fort Pierce, at Lake Placid, and in numerous smaller plantings at various other points in the State. The total number is estimated at 4,500. When Brevard County was revisited again in 1955 numerous additional trees, mostly *C. lepidophloia*, were found dead or dying from Clitocybe root rot.

[4]In addition to the single ornamental tree listed, Clitocybe root rot proved to be destructive in a ¾-acre planting of closely set stock plants of both the Indiarubber fig and the variegated variety grown under a lath shed for commercial propagation of the tips by "mossing" to stimulate rooting that the small number of plants surviving after a few years were destroyed and other crops planted (36). It is estimated that about 2,500 trees were killed in this planting.

TABLE 1.—(Continued)

Records of occurence			Records of isolations				
Scientific name	Common name	No. attacked	Year cultured	Condition of host	Locality		Fruited in culture
					Postoffice	County	
Ficus mysorensis Heyne	Mysore Fig	1					
Ficus pumila L.	Climbing Fig	1					
Ficus religiosa L.	Botree Fig	2	1936	Dead tree	Lake Alfred	Polk	Yes
			1936	Dying tree	Lake Alfred	Polk	Yes
Ficus sp. (S. P. I. No. 80569)	Fig	1	1943	Dead tree	Lake Alfred	Polk	Yes
POLYGONACEAE							
Antigonon leptopus Hook. & Arn.	Mountainrose Coralvine	6	1932	Dead vine	Gainesville	Alachua	Yes
			1933	Dead vine	Babson Park	Polk	Yes
Coccolobis uvifera L.	Common Seagrape	1					
MAGNOLIACEAE							
Michelia fuscata Blume	Bananashrub	2					
ANNONACEAE							
Annona cherimola Mill.	Cherimoya	4	1931	Dead tree	Cocoa	Brevard	Yes
Annona muricata L.	Soursop; Guanabana	1					
Artabotrys uncinatus Safford	Fragrant Tailgrape	1					
LAURACEAE							
Cinnamomum camphora Nees & Eberm.	Camphortree	20	1936	Declining tree	Candler	Marion	Yes
Cinnamomum cassia Blume	Cassiabark-tree	1	1942	Dying tree	Orlando	Orange	Yes
Persea americana Mill.	American Avocado	2	1933	Dead tree	Babson Park	Polk	Yes
Persea borbonia (L.) Spreng.	Redbay	1					
Persea littoralis Small	Shorebay	1	1943	Declining tree	Cocoa	Brevard	Yes
PITTOSPORACEAE							
Pittosporum tobira Ait.	Tobira Pittosporum	1					
HAMAMELIDACEAE							
Liquidambar styraciflua L.	Sweet Gum	2	1943	Dead tree	Gainesville	Alachua	Yes

ROSACEAE							
Cotoneaster pannosa Franch.	Silverleaf Cotoneaster	2	1943	Dead bush	Gainesville	Alachua	Yes
Cotoneaster sp.	Cotoneaster	2					
Crataegus sp.	Hawthorn	1					
Eriobotrya japonica Lind.	Loquat	16	1932	Declining tree	LaGrange	Brevard	Yes
			1939	Dead tree	Bonaventure	Brevard	Yes
Malus pumila Mill. (seedling)	Apple	1	1931	Dead tree	Artesia	Brevard	Yes
Prunus caroliniana (Mill.) Ait.	Carolina Laurelcherry	14					
Prunus persica (L.) Batsch (seedlings and trees budded on)	Peach	39[5]	1933	Dead tree	Babson Park	Polk	Yes
Prunus serotina Ehrb.	Black Cherry	3					
Prunus sp. (rootstock unknown)	Apricot	1					
Prunus sp.	Plum (Abundance)	1					
Pyracantha sp.	Firethorn	4					
Pyrus lecontei Rehd. (*P. communis* X *P. pyrifolia*)	Leconte and Kieffer Pears	2					
Pyrus pyrifolia (Burm.) Nakai	Sand Pear	20	1941	Dying tree	Quincy	Gadsden	No
Raphiolepis umbellata Schneid.	Yeddo Raphiolepis	1	1941	Dying bush	Monticello	Jefferson	No
Rosa multiflora Thunb. (rootstock)	Japanese Rose						
	Dr. W. Van Fleet[6]	1	1936	Dead bush	Leesburg	Lake	Yes
	Duchesse de Brabant	1					
	Etoile de France	1					
	Francis Scott Key	2					
	Lady Hillingdon	1					
	Paul Neyron	1	1936	Dying bush	Leesburg	Lake	No
	Radiance	2					
	Reine Marie Henriette	1	1936	Dead bush	Leesburg	Lake	No
Rosa sp. (rootstock unknown)	Rose						
	Climbing Maman Cochet	1	1936	Dead bush	Lake Alfred	Polk	Yes
	Marechal Niel	1					
	Reve d'Or	1					

[5]It is estimated that 575 additional trees in one 3½-year-old planting of about 1400 trees on peach stock and several others in another planting, both near Blanton, Pasco Co., have been attacked by Clitocybe root rot. The total number is estimated at 625.

[6]Clitocybe root rot was considered by Dr. W. B. Shippy to cause 75 per cent of the mortality of roses investigated by him in Florida. Only occasional instances of the disease have been recorded here.

TABLE 1.—(Continued)

Records of occurrence			Records of isolations				
Scientific name	Common name	No. attacked	Year cultured	Condition of host	Locality		Fruited in culture
					Postoffice	County	
Rosa sp. (rootstock and variety unknown)	Rose	12					
Spiraea vanhouttei Zabel	Vanhoutte Spirea	7					
Stranvaesia davidiana Decne	Chinese Stranvaesia	6	1943	Dead bush	Monticello	Jefferson	Yes
LEGUMINOSAE							
Acacia cornigera (L.) Willd	Bullhorn Acacia	1					
Acacia cyanophila Lindl	Blueleaf Acacia	1					
Acacia farnesiana (L.) Willd	Sweet Acacia	5	1933	Dying tree	Cocoa	Brevard	Yes
			1939	Dead tree	Lake Alfred	Polk	Yes
			1940	Dead tree	Malabar	Brevard	Yes
Acacia latifolia Benth	Broadleaf Acacia	1					
Acacia sp. (S. P. I. No. 106546)	Acacia	1					
Albizzia julibrissin (Willd.) Durazz	Silktree Acacia	1					
Albizzia lebbeck (L.) Benth	Lebbek	2					
Bauhinia purpurea L	Purple Bauhinia	11	1932	Dead tree	Courtenay, Merritt Island	Brevard	Yes
Bauhinia purpurea, var. *alba* Buch.-Ham	White Bauhinia	3					
Bauhinia tomentosa L	St. Thomas Bauhinia	1	1933	Dead tree	Babson Park	Polk	Yes
Bolusanthus speciosa Harms	Elephantswood	1					
Calliandra surinamensis Benth	Surinam Calliandra	2					
Cassia bicapsularis L	Senna	3					
Cassia fistula L	Goldenshower Senna	1					
Cassia fruticosa Mill	Senna	1					
Cassia nodosa Buch.Ham	Jointwood Senna	1					
Cassia siamea Lam	Siamese Senna	1	1933	Dead tree	Babson Park	Polk	Yes
Cassia siberiana DC	Siberian Senna	1					
Cassia surattensis Burm	Senna	1					
Ceratonia siliqua L	Carob	1					
Cercis canadensis L	Eastern Redbud	3					
Crotalaria mucronata Desv	Striped Crotalaria	2					

Erythrina crista-galli L.	Cockspur Coralbean	1					
Erythrina indica Lam.	India Coralbean	1					
Mimosa spegazzini Pirotta	Sensitiveplant	1					
Parkinsonia aculeata L.	Jerusalem-thorn	1	1937	Dying tree	Cocoa	Brevard	Yes
Peltophorum brasiliense (L.) Urban	Peltophorum	1					
Pithecellobium dulce Benth.	Guaymachil Apes-earing	16	1935	Dying tree	Malabar	Brevard	No
			1936	2 dead trees	Indianola, Merritt Island	Brevard	Yes
Poinciana regia Bojer	Royal Poinciana; Flamboyant-tree	7	1933	Dying tree	Artesia	Brevard	Yes
				Dead tree	Babson Park	Polk	Yes
Poinciana pulcherrima L.	Flowerfence Poinciana	1					
Wistaria sinensis (Sims) Sweet	Chinese Wistaria	2					
RUTACEAE							
Citrus limon (L.) Burm. f. (rootstock)	Rough Lemon						
	Calamondin Orange (*Citrus mitis* Blanco)	1	1935	Declining tree	Melbourne	Brevard	Yes
	Grapefruit (*Citrus paradisi* Macf.)	154[7]	1929	Living tree	Lake Alfred	Polk	Yes
			1930	2 living trees	Georgiana, Merritt Island	Brevard	Yes
			1931	Living tree	Highland City	Polk	Yes
			1933	Living tree	Georgiana, Merritt Island	Brevard	Yes
	Sweet Orange (*Citrus sinensis* Osb.)	70[7]	1932	Declining tree	Courtenay, Merritt Island	Brevard	Yes
	Tahiti Lime (*Citrus* aurantifolia Christm.) Swing	1					
	Tangerine (*Citrus reticulata* Blanco)	10					

[7]About 100 additional grapefruit and orange trees on rough lemon stock have been found attacked in addition to those itemized here.

TABLE 1.—(Continued)

Records of occurrence			Records of isolations				
Scientific name	Common name	No. attacked	Year cultured	Condition of host	Locality: Postoffice	Locality: County	Fruited in culture
Citrus paradisi Macf. (Seedling tree)	Grapefruit	1					
Citrus sinensis (L.) Osb. (Seedling trees)	Sweet Orange	7	1931	2 living trees	Rockledge	Brevard	Yes
			1932	Living tree	Rockledge	Brevard	Yes
MELIACEAE							
Melia azedarach L.	Chinaberry	2					
MALPIGHIACEAE							
Hiptage sp.		1					
Malpighia glabra L.	Barbadoscherry Malpighia	1					
EUPHORBIACEAE							
Acalypha wilkesiana Muell. Agr., Cl. Marginata	Painted Copperleaf	7	1933	Dead bush	Bradenton	Manatee	Yes
			1935	Dead bush	Melbourne	Brevard	Yes
			1939	Dead bush	Bonaventure	Brevard	Yes
			1939	Dead bush	Lake Alfred	Polk	Yes
Acalypha wilkesiana Muell Arg., Cl. Banana (*musaica*)	Painted Copperleaf	2					
Aleurites fordi Hemsl.	Tungoil-tree	104[8]	1937	3 dead trees	Gainesville	Alachua	Yes(2)
			1941	Dying tree	Lamont	Jefferson	Yes
Aleurites montana (Lour.) Wils.	Mu-oil-tree	1					
Euphorbia pulcherrima Willd.	Common Poinsettia	12	1925	Dead bush	Oakland	Orange	No
			1931	Dead bush	Cocoa	Brevard	Yes
Jatropha curcas L.	Barbadosnut	1	1932	Dying tree	Artesia	Brevard	Yes
Phyllanthus angustifolius Swartz	Leafflower	1					
Ricinus communis L.	Castorbean	1					
Sapium sebiferum (L.) Roxb.	Chinese Tallowtree	1					
ACERACEAE							
Acer rubrum L.	Red Maple	1					

ANACARDIACEAE							
Rhus copallinum L.	Shining Sumac	6	1936	Dead tree	Georgiana, Merritt Island	Brevard	Yes
			1937	Dead tree	Georgiana, Merritt Island	Brevard	No
Schinus terebinthifolia Raddi	Brazil Peppertree	21	1934	Dying tree	Palm Bay	Brevard	No
SAPINDACEAE							
Koelreuteria formosana Hayata	Flame Goldraintree	3					
Litchi chinensis Sonn.	Lychee	1[9]					
RHAMNACEAE							
Zizyphus jujuba Mill.	Common Jujube	1					
VITACEAE							
Vitis spp.	Grape	14					
TILIACEAE							
Grewia (?*occidentalis* L.)	Starflower Grewia	1	1943	Dead tree	Lake Alfred	Polk	No
Luehea seemannii Planch. & Triana	Whiptree	1					
MALVACEAE							
Hibiscus rosa-sinensis L.	Chinese Hibiscus	25					
Hibiscus syriacus L.	Shrubalthea	3					
Malvaviscus grandiflorus HBK.	Turkscap Waxmallow	58	1930	Dead bush	Cocoa	Brevard	Yes
			1932	Dead bush	Georgiana, Merritt Island	Brevard	No
Sida rhombifolia L.	Broomjute Sida	1					

[8]In addition to these trees it is estimated that 50 trees have died in one planting west of Gainesville, 150 in one at Lamont, and at least 25 each in plantings near LaCrosse, Brooker and Floral City. The total number is estimated at 375.

[9]As would be expected with the commercial planting of this tree of late years, Clitocybe root rot has appeared in a number. Cohen recently has reported 8 or 10 trees attacked in young plantings on Merritt Island, 15 young trees in a number of commercial plantings south of Sarasota, and an old tree in a dooryard planting at St. Petersburg. (Cohen, Mortimer, Clitocybe root rot of lychee. Citrus Industry 36(8): 11–15, 3 figs. Aug., 1955.)

TABLE 1.—(Continued)

Records of occurence			Records of isolations				
Scientific name	Common name	No. attacked	Year cultured	Condition of host	Locality		Fruited in culture
					Postoffice	County	
STERCULIACEAE							
Dombeya punctata Cav.	Burbon Dombeya	4	1931	Dead bush	Artesia	Brevard	Yes
			1934	Dying bush	Cocoa	Brevard	Yes
			1939	Dead bush	Bonaventure	Brevard	Yes
			1941	Dying bush	Cocoa	Brevard	No
Dombeya wallichi Benth. & Hook.	Scarlet Dombeya	1	1935	Dead bush	Melbourne	Brevard	No
Firmiana platanifolia (L. f.) Schott & Endl.	Chinese Parasol-tree	1	1944	Stump with living sprout	Gainesville	Alachua	Yes
TERNSTROEMIACEAE							
Camellia japonica L.	Common Camellia	2					
Gordonia lasianthus (L.) Ellis	Loblolly-bay	1					
CANELLACEAE							
Canella winterana (L.) Gaertn.	Cinnamon Canella	1					
COCHLOSPERMACEAE							
Cochlospermum vitifolium (Willd.) Spreng.	Yellowsilk Shellseed	1	1939	Dead bush	Lake Alfred	Polk	Yes
PROTEACEAE							
Grevillea robusta A. Cunn.	Silkoak Grevillea	8					
Macadamia ternifolia F. v. M.	Queenslandnut Macadamia	1					
MELASTOMACEAE							
Tibouchina glandulosa Cogn.		1					
Tibouchina semidecandra Cogn.		1	1933	Dead bush	Babson Park	Polk	Yes
LYTHRACEAE							
Cuphea hysoopifolia GBK.	Hyssop Cuphea	8					
Lagerstroemia indica L.	Common Crapemyrtle	2	1934	Dead tree	Melrose	Alachua	Yes

PUNICACEAE							
Punica granatum L.	Common Pomegranate	13	1933	Dead tree	Babson Park	Polk	Yes
COMBRETACEAE							
Quisqualis indica L.	Rangooncreeper	1					
MYRTACEAE							
Eucalyptus ficifolia F. v. M.	Scarlet Eucalyptus	2	1936	Declining tree	Lake Alfred	Polk	Yes
Eucalyptus globulus Labill.	Tasmanian Blue Eucalyptus	3					
Eucalyptus leucoxylon F. v. M.	White Ironbark Eucalyptus	1					
Eucalyptus multiflora Poir.	Beakpod Eucalyptus	33	1928	Dead tree	Cocoa	Brevard	Yes
			1937	Dead tree	Brooksville	Hernando	No
			1937	Dying tree	Brooksville	Hernando	Yes
Eucalyptus polyanthemos Schau.	Redbox Eucalyptus	1					
Eucalyptus rudis Endl.	Moitch Eucalyptus	3	1937	Dying tree	Brooksville	Hernando	No
Eucalyptus sideroxylon A. Cunn.	Mulga I. Eucalyptus	1					
Eucalyptus viminalis Labill.	Ribbon Eucalyptus	1					
Eucalyptus spp.	Eucalyptus	33[10]	1932	Dead tree	Brooksville	Hernando	Yes
Eugenia paniculata Banks ex Gaertn.	Brushcherry Eugenia	1					
Eugenia uniflora L.	Surinam Cherry; pitanga	11	1933	Dead bush	Windermere	Orange	Yes
			1936	Dying bush	Bonaventure	Brevard	Yes
Feijowa sellowiana Berg.	Feijowa	1					
Melaleuca leucadendron L.	Cajeput	1	1936	Dead tree	Bonaventure	Brevard	Yes
Metrosideros villosa Sm.	Rata	1					
Myrciaria cauliflora Berg.	Jaboticaba	1					
Myrtus communis L.	True Myrtle	5					
Psidium cattleianum Sabine	Cattley Guava; Strawberry Guava	11	1931	Dying tree	Lotus, Merritt Island	Bevard	Yes
			1933	Dead tree	Babson Park	Polk	Yes
Psidium guajava L.	Common Guava	102	1924	Dead tree	Cocoa	Brevard	No
			1925	Dying tree	Courtenay, Merritt Island	Brevard	No
			1933	Dead tree	Cocoa	Brevard	Yes

[10]A large number of additional trees of various species have died in a planting in the woods at Chinsegut Hill Sanctuary near Brooksville.

TABLE 1.—(Continued)

Records of occurrence			Records of isolations				
Scientific name	Common name	No. attacked	Year cultured	Condition of host	Locality		Fruited in culture
					Post office	County	
Psidium molle Bertol	Guisaro Guava	3					
Syzigium cumini (L.) Skeels	Jambolan	2					
Syzigium jambos (L.) Alston	Roseapple	4					
Tristania conferta R. Br	Brisbanebox Tristania	1					
CORNACEAE							
Cornus florida L	Flowering Dogwood	4	1941	Dying tree	Gainesville	Alachua	Yes
ERICACEAE							
Rhododendron indicum (L.) Sweet	Indica Azalea	32					
Rhododendron obtusum, var. *japonicum* (Maxim.) Wils. f	Kurume Azalea	1					
PLUMBAGINACEAE							
Plumbago capensis Thunb	Cape Plumbago	8	1936	Dead bush	Oakland	Orange	Yes
SAPOTACEAE							
Achras zapota L	Sapodilla	2	1932	Dead treee	Artesia	Brevard	Yes
Chrysophyllum cainito L	Cainito Starapple	1					
EBENACEAE							
Diospyros virginiana L. (rootstock for Japanese Persimmon (*Diospyros kaki* L.))	Common Persimmon	1					
OLEACEAE							
Jasminum dichotomum Vahl	Goldcoast Jasmine	1					
Jasminum gracile Andr	Australian Jasmine	1					
Jasminum mesnyi Hance	Primrose Jasmine	4	1940	Dead bush	Melrose	Alachua	Yes
Jasinum multiflorum Andr	Furry Jasmine	7	1935	Dying bush	Cocoa	Brevard	No
Jasminum officinale L	Common Jasmine; Poets Jasmine	1					

Jasminum officinale, var. *grandiflorum* (L.) Kobuski	Catalonian Jasmine	1					
Ligustrum amurense Carr	Amur Privet	17	1933	Dead bush	Rockledge	Brevard	Yes
Ligustrum japonicum Thunb	Japanese Privet	2					
Ligustrum lucidum Ait	Glossy Privet	2	1943	Dying bush	Quincy	Gadsden	Yes
Ligustrum spp	Privet	3					
Trachelospermum jasminoides Lem	Chinese Star Jasmine; Conferedate Jasmine	3					
LOGANIACEAE							
Buddleia asiatica Lour	Asian Butterflybush	1					
APOCYNACEAE							
Acokanthera venenata G. Don	True Bushmanspoison	1					
Nerium oleander L	Common Oleander	10	1932	Dying bush	Georgiana, Merritt Island	Brevard	No
			1937	Dying bush	Cocoa	Brevard	Yes
			1939	Dead bush	Lake Alfred	Polk	Yes
Tabernaemontana coronaria Willd	Crapejasmine Tabermontana	2	1933	Dead bush	Babson Park	Polk	Yes
Thevetia nereifolia Juss	Luckynut Thevetia; Yellow Oleander	1	1936	Dying bush	Lake Alfred	Polk	Yes
ASCLEPIADIACEAE							
Cryptostegia madagascariensis Hemsl	Madagascar Rubbervine	1	1935	Dead bush	Melbourne	Brevard	Yes
CONVOLVULACEAE							
Ipomoea setosa Ker	Brazilian Morningglory	1					
BORAGINACEAE							
Cordia obliqua Willd	Birdlime	1					
SCROPHULARIACEAE							
Verbascum thapsus L	Flannel Mullein	1					
BIGNONIACEAE							
Clytostoma callistegioides Bur. & Schum	Argentine Trumpetvine	1					
Jacaranda acutifolia Humb. & Bonpl	Sharpleaf Jacaranda	6	1936	Living tree	Lake Alfred	Polk	Yes
			1936	Dead tree	Lake Alfred	Polk	Yes

TABLE 1—(Continued)

Records of occurrence			Records of isolations				
Scientific name	Common name	No. attacked	Year cultured	Condition of host	Locality		Fruited in culture
					Postoffice	County	
Stenolobium stans Seem	Florida Yellowtrumpet	1					
Tecomaria capensis Seem	Cape Honeysuckle	1	1939	Dead vine	Bonaventure	Brevard	No
GESNERIACEAE							
Rhytidophyllum tomentosum (L.) Mart.		1					
ACANTHACEAE							
Pachystachys coccinea Nees	Blackstick Cardinalsguard	1					
Sanchezia nobilis Hook	Sanchezia	1					
Thunbergia grandiflora Roxb	Bengal Clockvine; Skyflower	2					
Thunbergia grandiflora, var. *alba* Hort	Bengal Clockvine; Skyflower	2	1936	Living vine	Gainesville	Alachua	No
			1937	Dead vine	Gainesville	Alachua	No
RUBIACEAE							
Gardenia jasminoides Ellis	Capejasmine	2					
Hamelia patens Jacq	Scarletbush Hamelia	1	1937	Dead bush	Bonaventure	Brevard	No
Ixora coccinea L	*Jungleflame Ixora*	3	1936	Dead bush	Cocoa	Brevard	Yes
CAPRIFOLIACEAE							
Abelia grandiflora (André) Rehd. (*A. chinensis* X *uniflora*)	Glossy Abelia	2					
Lonicera fragrantissima Lindl	Bush Honeysuckle	3	1943	Dead bush	Quincy	Gadsden	No
Viburnum odoratissimum Ker	Sweet Viburnum	2					
COMPOSITAE							
Montanoa hibiscifolia (Benth.) Sch. Bip.	Treedaisy	6					
Tithonia diversifolia Gray	Yucatan Tithonia	1					

ISOLATIONS OF THE CAUSAL FUNGUS

Clitocybe tabescens has been isolated consistently from a diverse array of trees, shrubs and vines attacked by mushroom root rot in various parts of Florida from 1924 to 1944. The only other organisms developing in these isolations were the ubiquitous bacteria, molds and soil-inhabiting fungi that may be expected to occur as contaminations. In most cases a large percentage of pure cultures of *C. tabescens* resulted, while in others a large percentage of contaminations occurred. The root-rot fungus is very slow to develop, usually requiring from one to two weeks, and occasionally longer, for growth to begin. As a result, if any contaminations are present, these organisms usually develop to the exclusion of the desired fungus.

After repeatedly isolating *C. tabescens* over a period of several years and carrying most of the isolates to the fruiting stage, there appeared to be no point in making further isolations, except in the case of new host plants or inoculated trees, though isolation of the fungus is always of value, especially when there is a possibility that *Armillaria mellea* may be involved. In many cases material was collected of root rot on new hosts while on extended field trips when facilities for making cultures were not available or other duties prevented. Nevertheless, *C. tabescens* has been isolated from root material of 156 plants, comprising 90 species from various parts of the State, as given in Table 1, the majority being from Brevard County, in which the writer was stationed.

In the writer's extensive cultural work with mushroom root rot in Florida, extending over a period of 20 years and conducted with a diverse array of trees, shrubs, and vines from many sections of the State, only two instances have occurred in which *A. mellea* was isolated and in both it was suspected when the roots were collected that this fungus rather than *C. tabescens* was involved. Both the trees in question occurred in areas of hammock forest on the Agricultural Experiment Station grounds at Gainesville. One was a living American hornbeam (*Carpinus caroliniana*) found in 1938; the other was a dying loquat (*Eriobotrya japonica*) found near the edge of the forest in 1941. During the course of these investigations this closely related root-rot fungus was observed to develop sporophores abundantly in hammock forests in the vicinity of Gainesville and Jacksonville during fall and early winter, but has not been observed to occur in central or southern Florida.

The general similarity of the cultural characters and rhizomorph production of these two closely related root-rot fungi stimulated the writer to make comparative cultural studies (35). *C. tabescens* is distinguished in culture principally by its more rapid growth, higher temperature range for optimum growth, and the readiness with which it usually fruits. The ends of the rhizomorphs developing upward and protruding above the surface of the agar in *C. tabescens* are short and relatively blunt at the tips and remain light-colored, while those in *A. mellea* usually are long and needle-shaped and become dark reddish-brown to blackish. Moreover, pure cultures of *C. tabescens* have consistently failed to show luminescence, whereas those of *A. mellea* usually exhibit it more or less strongly, at least when young and actively growing.

PATHOGENICITY OF THE FUNGUS

The parasitic nature of *C. tabescens* is clearly apparent from the readiness with which it attacks the most healthy and vigorously growing plants and the ease with which it may spread from one to another by root contact. Detailed data in regard to the rapid spread of this fungus and the high rate of mortality caused by it have been given by the writer for bananas and other exotic plants (29), for tung-oil trees (31), for citrus trees (37), and for windbreak and roadside plantings of the casuarinas (38).

It is clearly apparent that no mechanical injury is necessary for infection since it has been observed repeatedly in field investigations that, like *A. mellea*, *C. tabescens* can penetrate healthy roots readily through uninjured bark. The writer (28: 1938, 1939 and 1940) has confirmed this point by the successful infection of casuarina trees through the use of both pieces of naturally infected roots and pure cultures of *C. tabescens* grown on lengths of oak stems placed in contact with uninjured roots. Plakidas (14) also succeeded in producing infection with pure cultures of this fungus. He infected small Pineapple pear trees by placing in contact with the roots, after first wounding them, blocks of wood on which pure cultures were grown.

INCIDENCE OF THE DISEASE IN RELATION TO TIMBERED LANDS

Investigations on Clitocybe root rot in a number of States, notably Oklahoma, Missouri, Arkansas, Mississippi, Alabama, and Florida, have shown that the distribution of this disease coincides to a large extent with the location of areas where hardwood timber, particularly oak trees, occur or were prevalent prior to clearing. Wilcox (44), who, in 1901, reported *C. parasitica* (now known as *C. tabescens*) as the cause of a rhizomorphic root rot of orchard trees which was of common occurrence in parts of Oklahoma, stated that the distribution of this disease coincided with the location of the local timber belts. He stated further that the disease was largely or entirely confined to those orchards which had been planted on recently cleared timberland and that it had not been reported from orchards planted on strictly prairie soil. In addition to orchard trees (apple, cherry and peach), he found the fungus to be a common parasite and saprophyte on four species of oaks, but apparently confused the root rot caused by *C. tabescens* and *A. mellea*, both of which are now recognized as occurring in that State.

Faurot (8) reported observing root rot caused by toadstool-bearing fungi to be a very common and fatal disease of orchard trees in some sections of southern Missouri in 1902. He stated that planting apple trees on land that is free from stumps and giving it thorough cultivation seemed to very materially lessen the occurrence of this trouble, although it did occur on prairie soils where timber had never grown. Duggar (6, p. 471) stated that he had observed the Clitocybe root-rot fungus to occur abundantly during favorable seasons at Columbia, Mo., on roots of hickory and other deciduous trees but failed to observe its occurrence in orchards despite special effort to find it. The writer (16), who found this fungus to be the cause of a root rot of grapevines, as well as of orchard trees, in Missouri, observed it fruiting abundantly

during late summer and fall in an oak forest near Neosho, in the Ozark section. These reports indicate that *C. tabescens* is of widespread occurrence in this State and that, here also, it is native to timbered lands.

The same situation apparently prevails in Arkansas, according to Walker (43, pp. 29–32) and others (1), but in this State, according to these authors, mushroom root rot is attributed to both *C. tabescens* and *A. mellea*. The latter report states that both fungi have been under observation for a period of years and have been found commonly parasitizing a number of plants, including privet hedges, apple and peach trees, as well as grapevines.

In many sections of Florida the Clitocybe root-rot fungus has been found to exist along with the native trees, attracting no attention until

FIG. 11. Clusters of sporophores of *Clitocybe tabescens* developed at base of one of many turkey oaks (*Quercus leavis*) stumps on which the fungus fruited abundantly in cut-over woods following rainy fall season. Upturned cluster in foreground placed to show under side.

perhaps several years after the land was cleared and planted. In describing the loss of peach trees from this root rot in plantings on newly cleared, moderately high hammock land at Fulton, 4 miles from the mouth of the St. Johns River, in 1905, Hole (12) stated that the disease is found in hammock forest, primarily on oaks of mature growth, especially water oaks, and that it is found much more rarely in old cleared land.

The investigations of the writer have clearly shown that the distribution of Clitocybe root rot in Florida coincides definitely, to a large extent, with the location of areas where hardwood timber, particularly oak trees, occur or were prevalent prior to clearing. The disease appears to be absent in the typical flatwoods areas of the State, where the dominant vegetation is pine and saw palmetto, and oak and other harwood trees do not occur. In the prairie types of soils, where trees, particularly oaks, do not occur, as for example, the drainage

districts west of Vero Beach and Fort Pierce, and the muck soils of the Everglades, even the highly susceptible casuarina trees appear to be free from attack.

The investigations of the writer have shown further that the fungus may remain viable for several years in infected roots left in the land at the time of clearing, as a result of which these may serve as centers of infection to trees subsequently planted. Thus trees, even in fairly old orchards and other plantings, may contract the disease in spots where the existence of the causal fungus was not even suspected. The following examples are cited to show the general prevalence of Clitocybe root rot on areas of timberland prior to clearing.

In November, 1927, while driving through Sumter County after a prolonged rainy period, numerous clusters of the mushrooms of *C. tabescens* were observed for several miles along the highway north of Coleman, fruiting at the bases of stumps of turkey oak (*Quercus laevis*), the most common tree of the pine-oak forest characteristic of the Norfolk sand in this section of the State (Fig. 11). Later in the same month several successively developed clusters of the mushrooms of this fungus were observed on the roots of one of the dead live oaks (*Quercus virginiana*) and about old stumps of the same species in a citrus grove at Bonaventure.

Oak trees on residential properties at several points in Florida have been found attacked by Clitocybe root rot. As a rule, these trees have been fairly old or mature ones. In properties where such trees occurred *C. tabescens* frequently has been found fruiting at the bases of attacked living trees and around stumps following prolonged rainy periods. These trees and stumps commonly prove to be sources of infection for the root-rot fungus in ornamental plantings. In one such property at Lake Alfred, the owner has repeatedly lost highly-prized ornamental trees and shrubs over a period of several years from the ravages of this fungus. He informed the writer that he usually found infected oak roots in digging up specimens that had succumbed to root rot. In removing one specimen he reported having dug out a wheelbarrow load of oak roots. Infected oak and other hardwood roots also were found to occur abundantly under citrus trees attacked by root rot in groves at Lake Alfred and other points in Polk County. In fact, so prevalent is *C. tabescens*, both as a parasite and as a saprophyte, on native oak trees, and so commonly does the infection of various fruit, tung-oil, and ornamental trees, shrubs and vines take place from infected oak roots which have been allowed to remain in the land when cleared, that the name oak-root fungus, so commonly applied to *A. mellea*, is equally applicable to *C. tabescens*.

The writer was troubled since 1930 with occasional losses of various species of casuarina trees and ornamental shrubs planted about his residence in the Carlton Terrace Subdivision of Cocoa, where the soil is Lakewood fine sand characterized by a natural growth of sand pine (*Pinus clausa*), scrubby oaks and scrub hickory (*Carya floridana*), and of a pronounced droughty nature. After his house was built the vacant lots adjoining were cleared by cutting off the scrub and hoeing off the sprouts at intervals until the roots died. This practice was found to be bad later, as various ornamentals planted on land cleared in this

labor-saving fashion began to succumb to Clitocybe root rot. In digging out these as they died, oak and hickory stumps infected by *C. tabescens* often were found in close proximity to the dead ornamentals, and obviously were the sources of infection. In some instances the fungus fruited on old stumps and roots, thus disseminating spores for further possible infection. As a result of his experience, the adjacent lots were carefully grubbed before further plantings were made. However, even on the residential lot, where the soil was grubbed thoroughly prior to clearing, scalybark casuarina (*Casuarina lepidophloia*) trees were attacked occasionally by root rot over a period of several years. In the case of one tree that did not become attacked until quite large, in digging around the roots on the affected side the infection was traced to an old infected sand pine root that had been left in the soil of the adjoining lot in removing a large tree.

In the scrub adjacent to the clearing around the writer's residence an occasional sand pine has been observed to turn brown and die rapidly under conditions that indicated that bark beetles were not the primary cause or there was no evidence of their activity. Both small saplings and large trees were involved in this dying. Several such trees were dug up for examination over a period of years and they were found attacked by Clitocybe root rot. In one case the sudden death of a large, spreading sand pine beside the driveway was attributed to bark beetles that had attacked the tree. However, upon digging out this tree, 3 of the 6 large lateral roots near the surface of the soil, as well as the large taproot, were found extensively invaded by the root-rot fungus. Occasional small scrub oaks in the woods in the same vicinity also were observed to die from root rot. In one clump of these 5 small, recently dead myrtle oaks (*Quercus myrtifolia*) were found attacked. In adjoining residential properties, where the land had been cleared for several years, a good-sized tree each of scrub hickory (*Carya floridana*) and shore bay (*Persea littoralis*), which had been allowed to remain, were found to be seriously attacked by *C. tabescens*. In digging around the latter for surgical treatment two pieces of oak roots extensively rotted by this fungus were found close to the root crown. The death of a sand pine from Clitocybe root rot was noted also at Grand Island, as has that of two longleaf pine (*Pinus palustris*) saplings at Brooksville, 7 scattered shortleaf pine (*Pinus echinata*) saplings in an oak thicket at Quincy, and a large spruce pine (*Pinus glabra*) in a hammock forest at Gainesville. *C. tabescens* has been isolated on a number of occasions from infected roots of recently dead or dying pine trees. Native pine trees appear to be attacked by root rot but rarely, and then only when growing in association with oak or other hardwood trees.

The extremely high mortality of casuarina trees from Clitocybe root rot in situations where oak trees occurred prior to clearing or in close proximity to oak scrub has been discussed by the writer (38). In periodical inspections of the progressive dying of trees from this disease in a horsetail casuarina windbreak planting along one side of a citrus grove at Georgiana, Merritt Island, the dying of a few shining sumacs (*Rhus copallinum*) from root rot was noted in the dense oak scrub adjoining this planting at a point where most of the beefwood

trees had died. A single dead sumac was found in 1936 and the fungus isolated. In 1937 another sumac but 5 or 6 feet from this one was found to have died from root rot. In digging up this tree an infected root of scrub oak was found under it and *C. tabescens* was isolated from both. In 1939, 3 additional sumacs nearby in this area of scrub oak were found to have died from root rot, the fungus fruiting at the base of one. The periodic observations made in this and numerous other localities definitely establish the fact that in areas of hardwood forest or scrub where Clitocybe root rot occurs there is often a progressive spread of the fungus, with occasional dying of trees. However, young oak trees and oak scrub appear to be remarkably resistant to the disease and are killed but rarely.

Further definite evidence of the establishment and propagation of *C. tabescens* on timbered land prior to clearing has been afforded by observations made while clearing part of a 20-acre tract of land for a citrus grove. This was low-lying hammock land ranging from 2–5 feet above the level of the salt water in Sykes Creek at the head of New Found Harbor on Merritt Island. This fungus was found to occur on dead roots of large slash pine (*Pinus elliottii*) stumps, oak stumps and roots, and dead roots of southern waxmyrtle (*Myrica cerifera*) in 1938 and subsequently. In 1939, in clearing a low, mucky area, a living saw palmetto (*Serenoa repens*), with the older end invaded and partially rotted by *C. tabescens*, was grubbed out. Another nearby saw palmetto that had died a short time before also was found extensively invaded by this fungus. Both plants occurred near a clump of laurel oaks (*Quercus laurifolia*), one of which was dead from root rot. In 1941, in digging out a large, healthy-looking sand live oak (*Quercus virginiana*, var. *maritima*) at another point on this land, one of the lower roots was found attacked by *C. tabescens*, though the tree appeared perfectly healthy before digging it out. In a freshly uprooted large laurel oak observed in a hammock forest at Mayport later in 1941 some of the exposed roots were found attacked by root rot and *C. tabescens* was isolated. Other instances of laurel oaks attacked by Clitocybe root rot have been observed at City Point, Cocoa, Rockledge, Ocala and Gainesville. Instances of turkey oaks (*Quercus laevis*) attacked by this disease have been observed at Jacksonville, Leesburg, Lake Alfred, and Brooksville. In 1944, a living eastern hophornbeam (*Ostrya virginiana*) in a hammock forest at Gainesville, and a large, freshly uprooted loblolly-bay (*Gordonia lasianthus*) in the hammock forest at Gold Head Branch State Park were found attacked by Clitocybe root rot. A few years later, three young loblolly pine (*Pinus taeda*) trees were found to have died from Clitocybe root rot in an area of hammock forest near Gainesville.

Additional evidence was secured by the writer recently in the vicinity of Tallahassee that the incidence of *C. tabescens* increases rapidly after the clearing of forest land without removal of the roots. During the building of a residence on a heavily wooded lot many of the smaller trees (mostly oaks and other hardwoods) were cut from June to the end of July, 1951, to partially clear the back yard, without grubbing to remove the stumps and roots. Prior to clearing, no evidence of any trees dying from Clitocybe root rot was observed on this or adjacent

uncleared lots. When this property was visited again in June, 1952, a southern red oak (*Quercus falcata*) 12 in. D. B. H., in the middle of the rear part of this lot, was found to have died. On digging this out, *C. tabescens* was found to have extensively invaded a number of the main lateral roots and clearly was the cause of death. In grubbing roots from parts of the back yard at this time and again in April, 1953, the writer found a considerable number of dead ones invaded by *C. tabescens*. In October, 1955 two clusters of sporophores of this fungus were found to have developed from a stump and root at different points on the partially cleared lot adjoining to the south.

In digging around the yard of the writer's present residence, built in 1954 on a newly cleared wooded lot at Arlington on the east side of the St. Johns River at Jacksonville, a considerable number of oak roots and a few long-leaf pine (*Pinus palustris*) roots were removed on which *C. tabescens* was growing saprophytically. In later removing three living turkey oak (*Quercus laevis*), respectively 10, 11 and 12 in. D. B. H., the writer found that single roots on two trees and a number of them on a third one were attacked by *C. tabescens*.

The available evidence clearly shows that this root-rot fungus often becomes established in forested areas prior to clearing, and that it occasionally is a factor in the death of native trees, including both broadleaf trees and, occasionally, conifers. The fact that infected roots were found on a number of apparently healthy oak trees that were dug out to remove them indicates that Clitocybe root rot works rather slowly for several years before large trees succumb. On the basis of observations made in various parts of Florida, it appears that this root-rot fungus, when present, can spread quite rapidly as a saprophyte when trees are cut down and the roots left in the ground. The necessity for so much arduous digging and grubbing to determine the occurrence and extent of development of root-rot fungi on the root systems of trees is probably the main reason why so little is known in regard to the mode of infection and spread of *C. tabescens* and other root-rot fungi.

INCIDENCE OF THE DISEASE ON LAND CLEARED FOR MANY YEARS

It has been considered by all investigators of it that the incidence of mushroom root rot is directly correlated with plantings on newly cleared land and the impression prevails in the literature that these diseases are of little importance on land that has been cleared and cultivated for a few years. While it is undoubtedly true that such diseases are inclined to be less prevalent on such land, considerable evidence has been secured to show that, at least in Florida, Clitocybe root rot may be quite destructive at times even on land that has been cleared and under cultivation for a considerable number of years. As reported previously (37), in digging around 77 bearing citrus trees on rough lemon stock attacked by this disease in a number of groves at Lake Alfred, oak and other hardwood roots infected with *C. tabescens* were found under the majority of the trees. Since the groves in which these trees occurred ranged in age from 9 to 19 years, it is evident that roots left in the soil at the time of clearing can be a menace from the standpoint of transmission of the disease for a much greater number of years than is generally believed. Thus, the popular notion that

roots left in the ground soon decay and cease to serve as sources of infection is erroneous.

In this connection it may be remarked that cultivation in Florida citrus groves, as practiced of late years, is very shallow and does not tend to dislodge roots that may have been left in the ground. It is obvious that the majority of roots left in the land at the time of clearing are not infected. How so many become infected subsequently the writer has not been able to determine, especially as no evidence of mycelial strands or shoestring-like rhizomorphs extending through the soil has ever been detected in any of the large number of cases of Clitocybe root rot examined. It is quite likely, however, that the fungus develops with much greater readiness saprophytically than parasitically.

The writer (38) reported a surprisingly high mortality from this disease in species of *Casuarina* interplanted between many of the trees in a block of orange trees on sour orange stock at Courtenay, Merritt Island. This tract had been in grove for approximately 11 years and was cleared about 10 years prior to planting to grove, being first used for a nursery. Of a total of 91 6-year-old trees of *Casuarina lepidophloia*, 9, or 10 per cent, were dead or dying, while of a total of 66 5-year-old trees of *C. stricta*, 33, or 50 per cent, were dead or dying. These trees were mixed throughout the grove and the occurrence of the disease was not localized in any particular part. It is not known whether or not their infection was traceable to diseased foreign roots in the soil since the attacked trees were not dug out. In other properties at Courtenay and some at other points on Merritt Island the loss of casuarina trees at an early age has occurred occasionally in windbreak plantings around and through citrus groves on land that has been cleared for periods ranging from 20 to 50 years. In one instance uncleared areas of oak scrub occurred nearby, with a paved road intervening, while in others only groves of considerable size adjoined. It is thus apparent that, even on land that has been cleared for many years, there may be a gradual build-up or multiplication of the sources of infection through the development of the disease in planted trees, especially when highly susceptible kinds are used.

Although infected roots of oak and other trees left in the ground at the time of clearing have been found repeatedly to serve as sources of infection to trees subsequently planted, in many cases where trees attacked by Clitocybe root rot were dug up or around for examination no evidence of foreign roots was found. Occasional instances have been observed where individual trees have been attacked in residential and other properties where the land had been cleared for periods of 20 or more years. In some cases these trees had been growing for periods up to 20, and in one case even 35, years and the owners had no knowledge of the loss of other trees on these particular properties. An instance was reported (37) where 4 seedling sweet orange trees in a citrus grove at Rockledge were found attacked by Clitocybe root rot after attaining an age of from 40–45 years. The occurrence of the disease in such cases does not appear to be explainable on the basis of the roots of these trees coming into contact with infected foreign roots in the soil. In a number of instances where various trees in an early stage of root rot were examined the disease appeared to have

started at one side of the root crown. It can only be inferred that infection may at times develop in some way other than by root transmission, apparently from spores lodging in the leaf mold and litter about the bases, but this method of infection, though commonly assumed and perfectly logical, has never been demonstrated.

RELATION OF INCIDENCE OF THE DISEASE TO SOIL CONDITIONS

Since *C. tabescens* has been found to persist and spread in roots that have been left in cleared land and thereby provide sources of infection over periods of several years, it was thought that some correlation between the incidence of root rot and the pH reaction of the soil might be apparent, though there is no connection between the pH reaction of the soil and that of the tissues in which the fungus grows. Accordingly, in 1937, pH determinations were made of samples of soils, comprising a number of soil series, under beefwood trees (*Casuarina equisetifolia* and *C. lepidophloia*) and a variety of other trees and shrubs attacked by Clitocybe root rot. The readings of 5 samples of Lakewood fine sand ranged from pH 6.35 to 7.01, with another containing foreign calcareous material going 8.20. The readings of 4 samples of Gainesville sand (coquina phase) ranged from 6.31 to 8.25. The readings of 10 samples of Norfolk fine sand in an extensive windbreak planting of horsetail casuarina trees dying in wholesale fashion ranged from 5.64 to 6.54, with one running 7.56, and another sample from the shore of the Indian River, which contained shell fragments, running 8.00. The reading of one sample of newly cleared Portsmouth fine sand (hammock phase) was 4.15. All samples were taken from the top 6 inches. These readings cover the pH range of Florida's agricultural soils. These few determinations do not indicate any definite correlation between the pH reaction of the soil and the incidence of the disease.

Preliminary attempts were made by the writer (*28*: 1938, 1940) to determine the effect of the pH reaction of the media on the growth of both *C. tabescens* and *A. mellea* in pure culture, but without any particularly striking results. Both fungi on potato-dextrose-maltose agar exhibited a wide pH range on the acid side of the scale, starting with pH 3.9 in one series and 4.2 in another. *C. tabescens* grew well in general at all reactions up to 7.1 in one series and 8.7 in another, but fruiting was retarded on alkaline media, while *A. mellea* appeared to be distinctly intolerant of alkaline conditions and growth diminished after the neutral point was reached. However, the results obtained in these two series of cultures do not indicate that the growth of either fungus is sufficiently limited by the pH reaction of the medium to offer any practical application from the standpoint of control.

The impression prevails in the literature and textbooks dealing with the diseases of woody plants that root-rot fungi are more likely to occur on poorly drained soils than on well-drained or droughty ones. In many cases this viewpoint may result from the injury or death of roots from waterlogging, which frequently expedites the development of secondary organisms. However, the incidence of Clitocybe root rot in Florida has been found to be by far the greatest on well-drained, light sandy soils that are dominantly acid in reaction and commonly

subject to drought at frequent, and for often protracted, periods, though it also occurs on clay soils in western Florida and Alabama, which likewise are acid. But the fact that this disease usually is of infrequent occurrence on low hammock soils, which also are characterized by the prevalence of oak (particularly live oak) and other hardwood trees, indicates that some factor other than the occurrence of infected roots in the land is involved in its incidence. Such soils are heavier and characterized by a much greater average moisture content, and often by a fairly high water table during most of the year. It may be inferred that, with an abundance of moisture, the fungus should thrive. However, the question of aeration may be involved. Unless a good system of drainage is established, low hammock and other soils characterized by a high water table are subject to periodic waterlogging, in which case the fungus most likely would be killed. This is presumed to be the reason for the apparent absence of the disease in the extensive swamp hardwood forest areas of the State. Moreover, low hammock soils usually are neutral or alkaline in reaction, frequently being underlaid more or less closely by marl or shell.

Clitocybe root rot is unknown in the extensive plantings of casuarina trees and areas of natural reproduction of the horsetail casuarina from Miami to Homestead on the lower East Coast, some of the latter occurring on marl flats subject to periodic inundation by salt water. While oak trees occur to some extent in areas in this section, the soils of eastern Dade County, with few exceptions, are alkaline, being underlaid by oölitic limestone. Where this does not extend to the surface it is covered by a thin mantle of sand, peat, muck or freshwater marl. The relation of the calcium carbonate content of the soil to the reduced incidence of the disease in such situations is a point that merits further investigation.

The investigations by Gard (11) on the composition of the soils in different parts of France in which fruit trees, walnuts and grapes suffer from root rots by *A. mellea* and other fungi showed that such soils are very poor in calcium carbonate. This confirmed his opinion, expressed in a previous paper (10), that a deficiency of calcium carbonate in the soil is the chief factor that predisposes the cultivated walnut (*Juglans regia*) to attack by *A. mellea*. Gard correlated the incidence of *Armillaria* rot of this tree in various parts of France with the proportion of limestone in the soil and concluded that the spread of this disease in the past 20 years was due chiefly to a progressive reduction of the lime content of the soil. He found this disease to be rare in soils containing from 20–25 per cent of lime and absent in those with higher percentages, except where the water-holding capacity of the soil had been permanently increased. It is also of interest to note that Reitsma (15) found that *A. mellea* occurred most frequently on acid, sandy soils and least so on heavy alkaline or saline soils.

The influence of drought as a factor in reducing the resistance of plants to infection by the Clitocybe root-rot fungus also must be considered. In his study of root rot due to *A. mellea* on western white pine (*Pinus monticola*), Ehrlich (7) observed that on wet sites trees were less subject to infection than on moist or dry ones, and was of the opinion that moisture is favorable for the development of the tree, increasing its vigor and resistance to infection. The casuarinas,

which are extremely susceptible to root rot, are very tolerant of drought and well-established trees rarely exhibit symptoms of distress from lack of moisture, though citrus trees, with which they are commonly planted, frequently exhibit pronounced wilting over periods of several weeks. It should be borne in mind, however, that the casuarinas are essentially xerophytic, whereas *Citrus* is distinctly mesophytic. Although markedly resistant to drought, the casuarinas make their best growth on soils abundantly supplied with moisture. While their mortality from root rot is greatest on the droughtier soil types, it is difficult to evaluate drought by itself as a factor in predisposing to infection. It is of interest in this connection, however, to note the comparative freedom from Clitocybe root rot that prevails in plantings and natural reproduction along the lower East and West Coasts where oak trees are lacking and scarce and the land is a mixture of sand and shell and essentially very droughty. Those coastal plantings of the horsetail casuarina on land made by dredging sand and shell from under salt water, as at Davis Island in Tampa Bay and Sarasota on the West Coast, and at Miami Beach on the East Coast, appear to be characterized by complete freedom from root rot. It is possible that in such plantings both the alkalinity and salinity of the soil may be inhibiting factors to root-rot infection. Reitsma (15) observed that *A. mellea* was of infrequent occurrence on saline soils.

Field observations of the behavior and development of the Clitocybe root-rot fungus indicate that its growth and spread may be greatly checked, or even completely inhibited, during periods when the soil becomes decidedly dry but that it grows with great luxuriance and spreads with considerable rapidity during prolonged rainy periods. It is of interest in this connection to note that the writer has observed instances where people have unwittingly expedited the development of root rot on ornamental plants by watering them after they had begun to show evidence of decline, in the erroneous belief that the trouble was due to lack of moisture. One plant enthusiast, who was troubled by frequent losses of cherished plants from Clitocybe root rot over a period of several years at his place at Lake Alfred, reported that when he discontinued watering his plants so frequently during dry weather while getting ready to move them to a new locality, he observed a marked reduction in the loss from this disease. A case of remarkably high mortality from Clitocybe root rot in a stock planting of India-rubber fig (*Ficus elastica*) and its variety *variegata* grown, for propagation of the tips for the northern trade, under a lath-shed at a nursery near Apopka, where the plants were watered every day by means of overhead irrigation to keep the moss-layers wet, was reported by the writer (36).

An additional factor to be considered in connection with the incidence of Clitocybe root rot is the inherent susceptibility of the plant species to infection, which was previously discussed by the writer (39). The possibility of infection taking place at times in some way other than by root transmission also must be considered.

SUMMARY

This study summarizes the results of extensive investigations, conducted from 1924 to 1944 and intermittently subsequently, on

mushroom root rot of woody plants in Florida, caused by *Clitocybe tabescens*. This disease has been found of widespread occurrence and frequently quite destructive throughout a large part of this State, where it is definitely known to have occurred as far back as 1902, and apparently as early as 1885. In addition to native forest trees, it attacks tung-oil trees, a large variety of fruit trees, and ornamental trees, shrubs and vines, including many exotic ones.

A detailed account is given of the occurrence, distribution and economic importance of this disease and the symptoms on various kinds of plants are described, and its distribution in Florida mapped. A list is given of the known host plants in this State, comprising 213 species belonging to 137 genera and 59 families, together with the number of times the disease has been found attacking each. Species of *Casuarina* have proved extremely susceptible to Clitocybe root rot and these exotic trees have been found attacked much more frequently than any other. Peach trees also have proved highly susceptible. Citrus trees on rough lemon stock, tung-oil, the common guava, cypress-pine, and a number of other widely planted trees and shrubs also have proved very susceptible. In general, native trees and shrubs have been found vastly more resistant than many of the exotic ones.

Pure cultures of *C. tabescens* have been isolated from 156 plants, comprising 90 different species, from various parts of Florida, and the fungus carried to fruiting in most cases. During the cultural work carried out over a period of 20 years *Armillaria mellea* has been isolated from but two trees with root.

While *C. tabescens* commonly lives for years as a saprophyte, it has been found capable of attacking uninjured roots of the most vigorously growing plants and of spreading readily to adjacent ones, especially when closely planted. While the principal mode of transmission appears to be by root contact, the fact that trees are at times attacked when growing on land that has been cleared for 20 or more years and contains no dead roots indicates that infection also may occur at times in some other way, presumably from spores.

The pathogenicity of this fungus was demonstrated by the successful infection of casuarina trees, both through the use of pieces of naturally infected roots and pure cultures of the fungus grown on lengths of oak roots when placed in contact with uninjured living roots. In both cases the fungus was reisolated and carried to fruiting.

The distribution of Clitocybe root rot in Florida coincides definitely, to a large extent, with the location of areas where hardwood timber, particularly oak trees, occur or were prevalent prior to clearing. The disease appears to be absent in the typical flatwoods areas of the State, where the dominant vegetation is pine and saw palmetto, and in prairie soils. Numerous instances are reported to show how the fungus becomes established in hardwood forest areas prior to clearing, where it may be a factor in the mortality of native trees. The loss of planted trees and shrubs from root rot has been traced definitely in many cases to infected roots of native forest trees and other trees that remain in the soil when land is cleared, and these have been found to constitute sources of infection for a much greater number of years than is generally believed.

No correlation has been found between the incidence of Clitocybe root rot and the pH reaction of the soil in soils of a number of series which were tested, nor did the results obtained with pure cultures indicate that the growth of either *C. tabescens* or *A. mellea* is sufficiently limited by the pH reaction of the medium to offer any practical application from the standpoint of control measures.

The incidence of Clitocybe root rot in situations in Florida where hardwood timber occurs or was prevalent prior to clearing has been found to be by far the greatest on well-drained, light, sandy soils, which are dominantly acid in reaction and droughty in character, though it also occurs on clay soils in western Florida and Alabama, which likewise are acid. But the fact that the disease is of infrequent occurrence on low hammock soils, which usually are neutral or alkaline in reaction, and is unknown on the alkaline limestone lands in Dade County, despite the frequent occurrence of oak and other hardwood trees in places, indicates that some factor or factors other than the occurrence of infected roots in the land are involved in its incidence.

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A Preliminary Study of an Apparent Disease of Hemlock (Tsuga canadensis (L.) Carr.) in the Nursery

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During the summer of 1953, an apparent disease of the Canadian hemlock, *Tsuga canadensis* (L.) Carr., in Athens Co., Ohio was brought to the attention of the senior author. The trouble was spotted by Mr. George Carper, who owns and operates a landscape gardening nursery at Burlingham, which is located on U.S. Route 33 about midway between Athens and Pomeroy, Ohio. Mr. Carper reported that both hemlocks in his nursery and those which he had set in plantings for some of his customers were affected by the trouble, which was causing him considerable concern.

Symptoms and Prevalence.—The hemlocks examined in Mr. Carper's nursery showed a considerable amount of the trouble, although in most cases the plants were not completely dead. However, some branches bore only dead needles and the branches themselves in such cases were usually dead. One noticeable feature was that the lower branches and needles were the ones chiefly affected, the progress upward from this primary and principal infection being variable in amount and rate, sometimes engulfing the whole plant.

The appearance of infected needles varied from a slight light brown spotting on some parts of a needle to complete necrosis in others. Sometimes necrotic areas occurred as separate spots or a portion of a needle was wholly necrotic, while the remainder of the needle was green.

Affected hemlocks presented a very unattractive appearance with their more or less completely discolored and necrotic needles, or no needles at all, especially on the lower branches, and with some of these branches themselves dead.

This study has not been extended to other nurseries. According to Mr. Carper, other nurserymen were having the same trouble with their hemlocks. Observations made by the junior author on forest plantings failed to show the presence of this trouble there.

Isolation of a Causal Organism.—The pattern of origin and spread of infection in individual hemlocks indicated the probable presence of a more or less localized causal factor. Consequently, steps were taken to determine whether a pathogen could be isolated.

Attempts at isolation by means of tissue fragments of stems and needles following surface sterilization were not very successful. However, when infected twigs and needles were surface sterilized, and then placed in moist chambers for a few days, a white, fluffy fungus growth appeared. This fungus was observed to appear on several needles and in a circle in the xylem area of the cut ends of small twigs.

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The fungus was transferred to potato-dextrose agar slants where it developed a soft, white, cottony growth, which later became somewhat submerged. In spots a considerable amount of a viscous liquid, containing large numbers of spores appeared subsequently. The media assumed a deep pink color.

Testing the Pathogenicity of the Isolate.—The next step following the isolation of the fungus in pure culture was to determine whether or not it was pathogenic on the hemlock.

Spore suspensions were made in sterile water and the suspensions were applied by means of an atomizer. Both upper and lower surfaces of needles were sprayed. In some cases, the whole plant was sprayed, while in other cases only certain branches and their needles were sprayed, care being exercised to prevent the inoculum from coming into contact with the remaining branches and needles, which served as checks. Check plants and branches were sprayed with sterile water. In still other inoculations, branches were removed from the plant and then inoculated in the usual manner. In all cases, the test branches and plants, the latter being in pots, were placed in moist chambers following their inoculation.

Figures 1 and 2 show one of the potted test plants and the growth of fungus on an inoculated branch. The symptoms observed in the inoculated plants and twigs, namely, spotting and necrosis of needles, defoliation and killing of twigs, were quite similar to those observed in the nursery.

The fungus growth resulting from the inoculations was reisolated into pure culture and was found to be identical with the original isolate. The reisolate was then used in making more inoculations. These were also positive, and the same fungus was again isolated.

The above procedures were repeated several times, each series verifying the original positive results.

Identity and Characteristics of the Isolate.—The authors identified the isolate as a species of *Cephalosporium*. One of the distinguishing characteristics of this genus is the habit of bearing conidia in clusters, sometimes called "cephalosporia" (spore balls), which are, in turn, surrounded by a rather copious amount of a somewhat viscid secretion. This characteristic was quite evident in the cultures of this fungus as mentioned earlier in this paper. The conidia are borne one at a time at the tip of the conidiophore and are pushed aside as new ones form. But the viscous secretion keeps them from being immediately disseminated, hence the formation of the cephalosporia as mentioned above.

Discussion.—Many species of *Cephalosporium* occur as saprophytes and soil inhabitants. However, some species are known as plant pathogens. Denyer (1953) described a Cephalosporium canker of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) from British Columbia. In our case the pathogen may live primarily as a saprophyte and become parasitic when the vigor of the tree is reduced. Christensen (1937) also reported a Cephalosporium disease. He found a species of *Cephalosporium* attacking balsam fir (*Abies balsamea* (1.) Mill.) in Minnesota and Wisconsin. Similarly Goss and Frink (1934) reported a Cephalosporium disease occuring on *Ulmus americana* (L.). In the

FIG. 1. A potted hemlock seedling used for inoculation experiments.

FIG. 2. A branch tip of hemlock, showing mycelial growth of the fungus on the needles. Inoculated with a spore suspension.

light of our results the isolate may be regarded as a pathogen on hemlock, at least in the nursery. As reported earlier in this paper, numerour observations have failed to show natural infection by this fungus in forest plantings of hemlock.

As yet it can not be stated that the source of inoculum is found in the soil. However, observations to the effect that the first symptoms and the predominance of symptoms and injury are found in the lower branches of the infected plant indicate infected soil as the probable source of primary inoculum. Wounds incurred during transplantation possibly serve as entrances.

Further work is needed to determine the distribution and importance of this nursery trouble and its possible means of prevention and control.

Summary.—An apparent disease of hemlock (*Tsuga canadensis* (L.) Carr.) was noted in an Athens County, Ohio, nursery. Infected plants showed browning and necrosis of needles, followed by defoliation, especially on the lower branches. Twigs and branches also were killed.

From infected twigs and needles, a fungus was isolated, which, through the use of Koch's Postulates, was found to be pathogenic on hemlock.

This fungus was identified as an imperfect fungus belonging to the genus *Cephalosporium.*

Infection was not observed on forest plantings of hemlock.

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Studies in Autotetraploids of Linseed (Linum usitatissimum L.), II. Morphology and Cytogenetics

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INTRODUCTION

The quantitative changes brought about by autopolyploidy generally lead to gigantism of the plant and its various parts. Thus in plants which are grown for their vegetative parts as their economic products (many vegetables and fodders, etc.) tetraploidy has been of great importance. Sometimes a significant increase in the desirable chemical composition of vegetative parts is reported, viz. nicotine content in *Nicotiana* (Noguti, Oka and Otuka, 1940) and sugar content in sugar beet (Peto and Hill, 1943). Autopolyploids have also been useful in floriculture and horticulture where big flowers, extended flowering season, and complete sterility are highly desirable characters. But in plants whose seeds constitute economic products—as in cereals, pulses, and oil crops—antopolyploidy has so far been of little use because they are usually highly seed sterile.

The causes of seed sterility in autopolyploids are still not quite clear. It seems that the duplication of the genome complement, apart from meiotic irregularities, also brings about a shift in its physiological equilibrium which may in turn be reflected in changes of a variety of characters—morphological, physiological, and embryological—essential for normal healthy seed setting of the plant.

Most efforts to study the problem of seed sterility in autopolyploids were concentrated on cytological abnormalities in pollen mother cells. It was thought that the cytological condition of male and female organs would be identical and that the study of meiosis in pollen mother cells was enough to understand the cytological condition of the ovule too. But low seed setting in apparently highly pollen fertile tetraploid plants of some species indicated that female sterility may not be identical with its male counterpart. Apparently limited studies have been made of megasporogenesis, embryo-sac formation, fertilization, and post-fertilization stages in induced autotetraploids. Einset (1944) reported some observations on this line in an induced autotetraploid of *Lactuca sativa* which indicated that in this tetraploid meiosis was seriously upset in a considerable percentage of ovules. After the present author reported various pre-fertilization abnormalities in the female side in autotetraploid *Linum usitatissimum* (Pandey, 1950), investigations in autotetraploids of *Brassica campestris* var. *Toria* (Parthasarathy, 1953) have revealed similar phenomena. Low seed setting in autotetraploids of these species is largely due to pre-fertilization abnormalities leading to abnormal or degenerate embryo-sacs, and in *Brassica campestris* var. *Toria* even to non fertilization of a great number of apparently normal ovules. In tetraploid *Trifolium*

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pratense (Pandey, 1955b) a comparatively higher degree of failure of polar fusion at the time of flower opening occurred in tetraploids than was common among diploids; in several cases the two polars were distinctly apart even a few days after flower opening. However, the poor seed set was mainly due to post-fertilization disturbances leading to the collapse of the developing endosperm. Therefore, a complete study of the development of male and female organs was necessary for the proper understanding of the phenomenon of sterility in autotetraploids.

The physiological effects of autopolyploidy in *Linum usitatissimum* on growth rate have already been studied (Pandey, 1956). The present paper deals with investigations concerning comparative morphology, cytology, and breeding behavior of diploid and tetraploid strains of *L. usitatissimum* with special reference to the problem of seed sterility in autotetraploids.

TECHNIQUES

1. *Anatomy of the stem.*—Stem pieces of 3 cm. length were taken from plants of the same age at a fixed distance of 5 cms. from the cotyledonary node and fixed in F.A.A. (Darlington and LaCour, 1950). Transverse sections of the stem were cut by hand razor and stained in safranin and fast green.

2. *Pollen germination and pollen tube growth.*—Styles along with stigma were detached from the ovary and were fixed in acetic-alcohol at 1, 3, 6, 9, and 24 hours after pollination. On an average five styles were collected per treatment. Styles were then macerated for about 10–15 minutes in 45 per cent acetic acid at 50°C. and then transferred in a watchglass containing distilled water. They were stained in a combination of Acid Fuchsin—Light Green (1:1) for 5 hours, cleared in 80 per cent lactic acid in water and mounted in glycerine. A gentle pressure on the cover slip spread out the stylar tissue into a thin layer. The preparations were sealed with melted paraffin.

3. *Development of the embryo.*—Ovaries were fixed after anthesis before pollination as well as at 6, 24, 48, 72, 96, and 120 hours after pollination in Karpechenkos' modification of Navashin's fluid. They were slightly cut at the top and the bottom and also punctured at several places by a fine needle to facilitate the penetration of the fixative inside the ovary wall which is rather tough in linseed. The ovaries were subsequently processed by the paraffin method (Johansen, 1940). Longitudinal microtome sections were cut at 13-17 μ in different planes. The sections at 15μ with the top and the bottom of the capsule parallel to the edge of the razor blade, proved to be most suitable for observations. Among the different stains tried, Iron Haematoxylin and Safranin gave best results.

4. *Cytological technique.*—(a) *Meiosis:*—Anthers of young buds were smeared in 1 per cent aceto-orcein without prefixation, between 10 and 11:30 a.m.; the fixed buds gave deeper staining of the cytoplasm. But whenever necessary, buds were fixed in acetic-alcohol for 24 hours and changed to 70 per cent alcohol for storage. Anthers from these buds were softened in 45 per cent acetic-acid for 5–8 minutes and smeared with an unplated needle in aceto-orcein of 0.5 per cent strength. The least possible pressure was applied to the cover slips

to maintain the configuration of the chromosomes. The slides were made permanent by McClintock's Schedule (1929).

(b) *Mitosis*:—The time of maximum division was ascertained by root-tip smears. Root tips were fixed in Belling's modified Navashin and other CRAF solutions. Paraffin blocks were prepared with the

Fig. 1

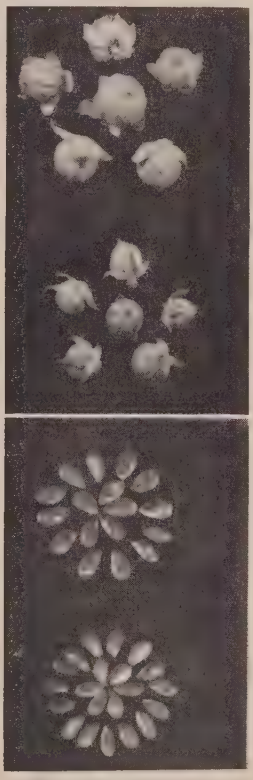

Fig. 2—Upper.

Fig. 3—Lower.

PLATE 1.—Figs. 1–3.—Comparative size differences between diploid and tetraploid *Linum usitatissimum*. Fig. 1. Plant. Fig. 2. Fruit. Fig. 3. Seed. Tetraploids (on the left side) are significantly bigger than diploids.

normal method (Darlington and LaCour, 1950). Sections were cut at 10μ, stained in iodine-gentian violet and mounted in Canada balsam. Considerable difficulty in staining was experienced owing to the presence of cytoplasmic inclusions which resulted in poor preparations.

OBSERVATIONS

1. *Morphological Characters*:—There was a general increase in the size of different parts in tetraploid plants including flower, flower parts, plant height, capsule, and seed (Plate 1, Fig. 1). There was also an

increase in the number of branches in tetraploids. The size of pollen grains and stomata was significantly increased showing that the cell size in tetraploid plants of linseed increased with the chromosome number. In some parts like stamens in RR 63, with increase in the size of anthers the size of filaments decreased. Pollen fertility generally ranged from 70–90 per cent in tetraploids and 98–100 per cent in diploids. The average size of capsule was notably greater in tetraploids but the average weight was considerably lower than in diploids (Plate 1, Fig. 2). It was due to the aborted, shrivelled or undeveloped nature of many seeds in tetraploids, the thousand seed weight of which was either slightly lower or just equal to that of diploids. The number of seeds per capsule varied from 0–6 in tetraploids and 9–10 in diploids. The size of well-developed seeds was considerably greater in tetraploids —a character of great economic importance (Plate 1, Fig. 3). This advantage in tetraploids was, however, generally suppressed by the shrivelled nature of the seeds, so much so that the average weight of the seed hardly crossed the diploid level. The range of variability in the morphological characters like height of the plant, number of branches, pollen fertility, and number of seeds per capsule was greater in tetraploid lines than in the respective controls. This was a hopeful character as it enhanced the scope of improvement by selection.

2. *Anatomy of stem with special reference to fiber bundles.*—Generally doubling of chromosomes did not affect the cellular organization of the various tissues. Minor differences in the arrangement of cells within tissues and some quantitative differences in the amount of tissues were, however, commonly found.

Transverse sections of linseed stems showed a slight general increment in the size of cells in tetraploids. Conspicuous differences between the tissues of diploid and tetraploid stems were noted in regard to the following characters (Plate 2, Figs. 1 and 2):—

(a) The cortex consists in diploids of several layers of medium sized collenchymatous cells containing chloroplasts, whereas in tetraploids the collenchymatous cells are narrower, compressed, and irregular in shape.

(b) Fiber bundles in diploids are compact and generally continuous with little irregular boundary. In tetraploids, fiber bundles are often distinctly separated from each other by groups of parenchymatous cells. The fiber band is narrower and very irregular in boundary. The fiber cells are loosely packed with more parenchymatous cells between them. The size of fiber cells as observed in transverse section is more variable in tetraploids than in diploids. In certain areas fiber bundles are compressed into a single layer of loose fiber cells. The number of cells in a group varies, the average being 30 cells in diploids and 20 cells in tetraploids.

(c) Xylem vessels are radially very irregular in tetraploids and sometimes protrude into the fiber band which is reduced to a single layer of loose cells.

(d) The hollow pith occupies a relatively greater area in tetraploids than in diploids.

3. *Floral biology.*—Anthesis in tetraploids started generally at 7 a.m., a little later than in diploids. Dehiscence of anthers and

opening of flowers took place simultaneously in diploids but in tetraploids anther dehiscence in many flowers started after opening of the flower. In diploids nearly all flowers opened in the morning while in tetraploids a good number of them opened during noon and sometimes even in the afternoon.

In tetraploids two types of anther dehiscence were recorded:—(a) in some plants (about 10 per cent) at the time of anthesis filaments with the anthers stretched away from the face of the stigma. In such cases only a few pollen grains, if any at all, were found on the stigma. The production of seeds in these plants was considerably reduced owing

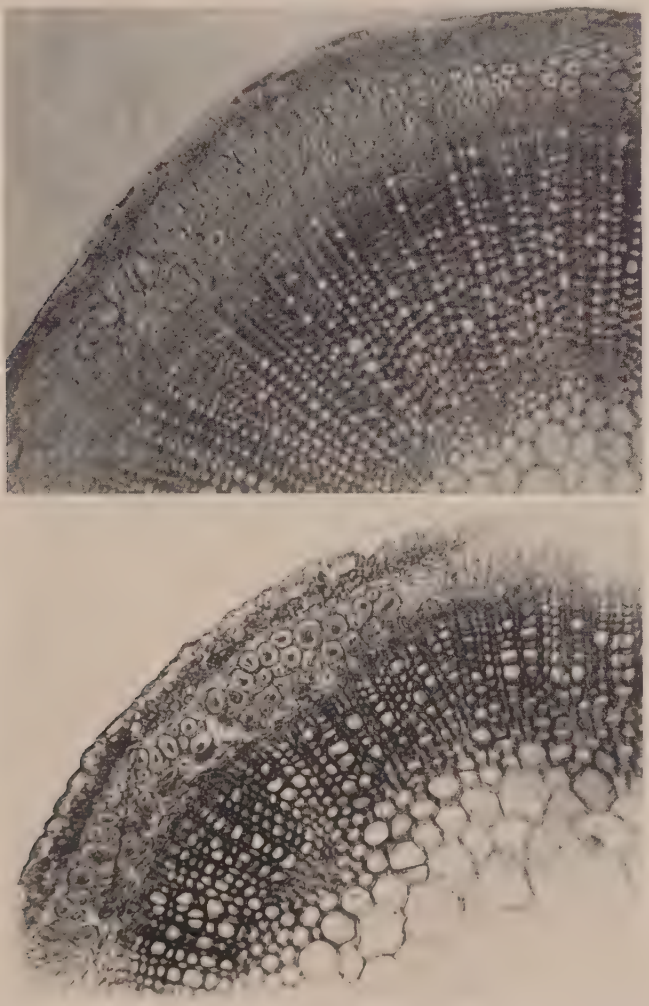

PLATE 2.—Transverse section of stem of diploid (Fig. 1) and tetraploid (Fig. 2.) *Linum usitatissimum*. The fiber band is narrower and irregular and the fiber cells may be loose in the tetraploid.

presumably to the lack of sufficient number of pollen tubes available for fertilization. (b) The other was the usual type in which anthers gripped the stigma from all sides, thus covering it with a large number of pollen grains. This is the normal method of dehiscence for diploids and most of the tetraploid plants.

By pollinating diploid and tetraploid stigmas at various intervals it was found that the stigma of the tetraploid plant remained receptive for about 20 hours (commencing from the evening prior to flower opening) whereas the corresponding period of receptivity in the case of diploids was about 17 hours.

TABLE 1.—*Comparative seed setting in tetraploids, with different modes of pollination: Natural selfing (flowers bagged before opening), Open pollination (no bagging) and Hand pollination.*

Types	No. of flowers	No. of capsules	Average size of capsules in mm.	No. of seeds	Average No. of seeds per capsule
			Natural Selfing		
RR 63	17	17	7.8×7.3	26	1.5
RR 68	18	18	10.3×8.6	32	1.8
			Hand Pollination		
RR 63	28	28	9.0×8.2	72	2.6
RR 68	8	7	11.0×9.3	16	2.3
			Open Pollination		
RR 63	24	24	8.7×8.2	51	2.1
RR 68	20	20	10.1×8.8	38	1.9

TABLE 2.—*Seed setting in tetraploid (T) and diploid (D) crosses: T×T, T×D, D×T.*

Crosses	No. of crosses	No. of capsules developed	Average size of the capsules in mm.	No. of seeds set
T X T				
RR 63 × RR 68...	20	2	9.3×8.4	4
RR 68 × RR 63...	20	17	10.9×9.1	43
RR 63 × RR 39...	18	8	7.3×6.6	2
RR 68 × RR 39...	14	12	10.6×8.9	28
T X D				
RR 63 × RR 39...	23	12		3
RR 68 × RR 63...	24	11	Largely	0
D X T			varying	
RR 39 × RR 63...	12	3	sizes.	0

4. *Crossability*:—The comparative observations in tetraploids on natural selfing (flowers bagged before opening), open pollination (no bagging), and hand pollination are given in Table 1. The result of hand pollination seems to be better than that of either natural selfing or open pollination.

Tetraploid × Tetraploid:—

In reciprocal crosses among tetraploids, when RR 63 was used as the female parent, very few seeds were produced; even the number of capsules developed was very low (Table 2). It may be mentioned

that all tetraploids used for crossing had 60 chromosomes and had about 80 per cent pollen fertility as observed under the microscope in acetocarmine mounts.

Tetraploid × Diploid and Diploid × Tetraploid:—

From 59 reciprocal crosses between tetraploid and diploid, only 3 seeds could be obtained, though partial development of capsules took place in as many as 26 capsules. These capsules exhibited largely varying sizes (Table 2). The 3 seeds obtained from 4n × 2n crosses were plump and mottled, and resembled those of the diploid in size. No seed was obtained in crosses 2n × 4n although a few capsules developed to a small extent. The stimulation of the ovary indicated that pollen germinated and entered the style but seed development failed either due to failure of pollen tubes to reach the embryo-sac thus leaving the egg unfertilized or, after fertilization, due to abortion of the triploid zygote or embryo.

5. *Pollen germination and pollen tube growth.*—(i) Pollen germination:—The number of germinated and ungerminated pollen grains was counted on stigmas of both the diploid and the tetraploid at different intervals after pollination. Plants used in pollination had about 90

TABLE 3.—*Percentage of germinated and ungerminated pollen grains at 1, 3, 6, 9, and 24 hours after pollination.*

Types	3 hours		6 hours		9 hours		24 hours	
	Germinated	Ungerminated	Germinated	Ungerminated	Germinated	Ungerminated	Germinated	Ungerminated
2n	76.7	23.3	83.3	16.5	91.9	9.1	92.9	7.1
4n	55.6	44.4	70.9	29.1	75.0	25.0	76.2	23.8

per cent pollen fertility in tetraploids and about 100 per cent in diploids. Figures in table 3 represent percentage values calculated from observations on more than 750 pollen grains obtained from 5 stigmas in each treatment.

In diploids 76.7 per cent of the pollen grains germinated during the first three hours after pollination and subsequently only 16.2 per cent germination took place. But in tetraploids, only 55.6 per cent of the pollen grains (including sterile grains) germinated within the first three hours after pollination and 20.6 per cent of pollen grains germinated during the later hours. Twenty-four hours after pollination when germination had practically ceased in both diploids and tetraploids, it was found that whereas only 7.1 per cent of the pollen grains remained ungerminated in the diploid, the corresponding percentage in the tetraploid was 23.8 (Plate 4, Fig. 1). This clearly indicated that some of the apparently fertile pollen grains in tetraploids (13.8 per cent) were unable to germinate.

(ii) kinds of pollen tubes:—

Observations were recorded on the number of pollen tubes present in the styles of the 2n and 4n at 3, 6, 9, and 24 hours after self-pollination. A majority of the pollen tubes was found to be abnormal

in shape at their tips both in the 2n and 4n; four distinct types were recorded (Plate 3, Figs. 1, 2, 3 & 4):

(a) Tips wedge-shaped and intact,
(b) Tips swollen and burst,
(c) Tips having roundish swelling,
(d) Tips quite normal with no swelling.

Of these types, the one with wedge-shaped, intact tips (a) was found to occur most frequently (Plate 4, Fig. 2). These shapes of the tubes appeared from the very beginning of pollen germination. Most of the pollen tubes from their emergence formed wedge shaped tips but many of them were found to burst before they proceeded any length. Some of these abnormal intact tips were observed even down to the base of the style. Table 4 shows the percentage of four types of pollen tubes observed in styles of diploids and tetraploids.

Though these abnormalties in pollen tubes were common to both diploids and tetraploids, the percentage of tubes which burst either in the very beginning or during the course of their growth in the stylar tissue was distinctly greater in tetraploids than in diploids (Plate 4, Fig. 2).

6. *Embryology.*—(i) Megasporogenesis and megagametogenesis (stages at the time of anthesis):—(a) Diploid:—Microtome sections of ovaries disclosed that embryo-sacs in almost all ovules had attained the eight-nucleate stage at the time of flower opening and were ready to receive the pollen tube. Generally nine out of ten ovules in a capsule contained fully developed eight-nucleate embryo-sacs. The size of the ovules varied very little, only one of the ovules in a capsule was sometimes a little smaller than the others. In about 10 per cent of the ovules examined it was observed that the two polar nuclei had not yet fused and were lying close to each other. The embryo-sac was oval in shape and occupied a comparatively small space in the huge mass of nucellus. The tapetum usually consisted of only one layer of compact cells and was well differentiated from the nucellus.

(b) Tetraploid:—In tetraploids, the condition was very different from that in diploids. The ovules were larger and the embryo-sacs showed varying shapes and sizes. The tapetum was thicker than in diploids and had larger cells. Only a very small percentage of the ovules seemed to contain more than three nuclei. In most instances the embryo-sac was far from completely formed. About 50 per cent of the ovules examined contained only two nuclei and another 20 per cent had three nuclei. Some of these nuclei represented the megasporogenetic and other megagametogenetic stages. Ovules with three nuclei represented the stage corresponding to the tetrad stage of the megasporogenesis except that three spores were present instead of the usual four. Such a 'triad' of spores presumably resulted from the first division of the egg-mother-cell followed by another division in only one of the products of the first division. Embryo-sacs with two nuclei were bigger and represented the two-nucleate stage; alongside most of them a few of the degenerating, compressed nuclei of the upper megaspores were visible.

Variation in the size of the nuclei comprising the 'triads' might be due to their having unequal chromatin contents. Although meiosis

PLATE 3.—Pollen grains and pollen tubes of diploid and tetraploid *Linum usitatissimum*. Four kinds of pollen tubes are observed in diploid as well as tetraploid: Fig. 1. Normal. Fig. 2. Tips wedge-shaped and intact. Fig. 3. Tips with roundish swelling. Fig. 4. Tips swollen and burst. Generally pollen grains of tetraploid have four germ pores (Fig. 5.) and that of diploid have three (Fig. 6.). The Tetrad pollen grains in tetraploid. Fig. 7. Normal. Fig. 8. Octad. Fig. 9. Hexad.

in the megaspore mother cell could not be studied and quantitative data on these aspects were not obtained, observations on later stages, however, suggested that the first meiotic division of the megaspore mother cell took place and gave rise to two unequal nuclei. Of these perhaps only the bigger nucleus divided again to give two unequal nuclei. Thus a 'triad' of unequal sized spores resulted.

In all two-nucleate embryo-sacs, each of the two nuclei appeared to contain a vacuole. This unusual condition of nuclei indicated a degenerating condition of the chromatin.

(ii) Stages 24 hours after pollination:—(a) Diploid:—At twenty-four hours after pollination an endosperm tissue usually containing 14–20 nuclei had formed by free nuclear division of the endosperm mother cell. In a few ovules, endosperm with a smaller number of nuclei was also observed. The zygote was either in the resting stage or had divided twice producing four cells. At this stage endosperm usually occupied a major portion of the embryo-sac which had also increased in size.

TABLE 4.—*Percentage of different types of pollen tubes in the styles of the diploid and the tetraploid. These figures were calculated from observations on 200 pollen tubes from the flowers collected 24 hours after pollination.*

Types	Wedge-shaped intact	Swollen and burst	With roundish swelling	Absolutely Normal
2n.....	56.2	12.5	6.2	25.0
4n.....	52.9	27.7	8.3	11.1

(b) Tetraploid:—In tetraploids, even 24 hours after pollination, the condition of most ovules was not much different from that observed at the time of anthesis before pollination. Ovules with different numbers of nuclei, varying from a single megaspore mother cell to three-nucleate embryo-sac stages, were observed. In some of the embryo-sacs, a pollen tube was clearly observed to have entered nearly one-third of the embryo-sac, but the tube had remained unburst. This was probably because the embryo-sac had not reached the right stage of development and had therefore failed to stimulate the tip of the pollen tube to burst on contact with it. The few pollen tubes traced in embryo-sacs were all of the straight, unswollen normal type.

(iii) Later stages of development:—Diploid and tetraploid:—The development of the embryo was normal in diploids; 72 hours after pollination, embryos with differentiated cotyledons, hypophysis, and and suspensor were noticed.

In tetraploids, out of 10 ovules in a capsule generally one or two were found developing normally, the development being usually slower than in diploids. The pro-embryo stage were rarely found before 72 hours after pollination.

7. *Cytology.*—(i) Diploid:—The diploid varieties of *L. usitatissimum* used in the present study had invariably $2n=30$ chromosomes. Meiosis in these types was quite regular with the formation of fifteen

bivalents at first metaphase. The separation of anaphase I was found to be perfectly regular. The metaphase II plates consisted of fifteen chromosomes each, and anaphase II and tetrad formation were normal. Pollen fertility in diploids was 98–100 per cent.

(ii) Tetraploid:—Attempts were made to determine the chromosome number of as many tetraploid plants as possible. Though meiosis was

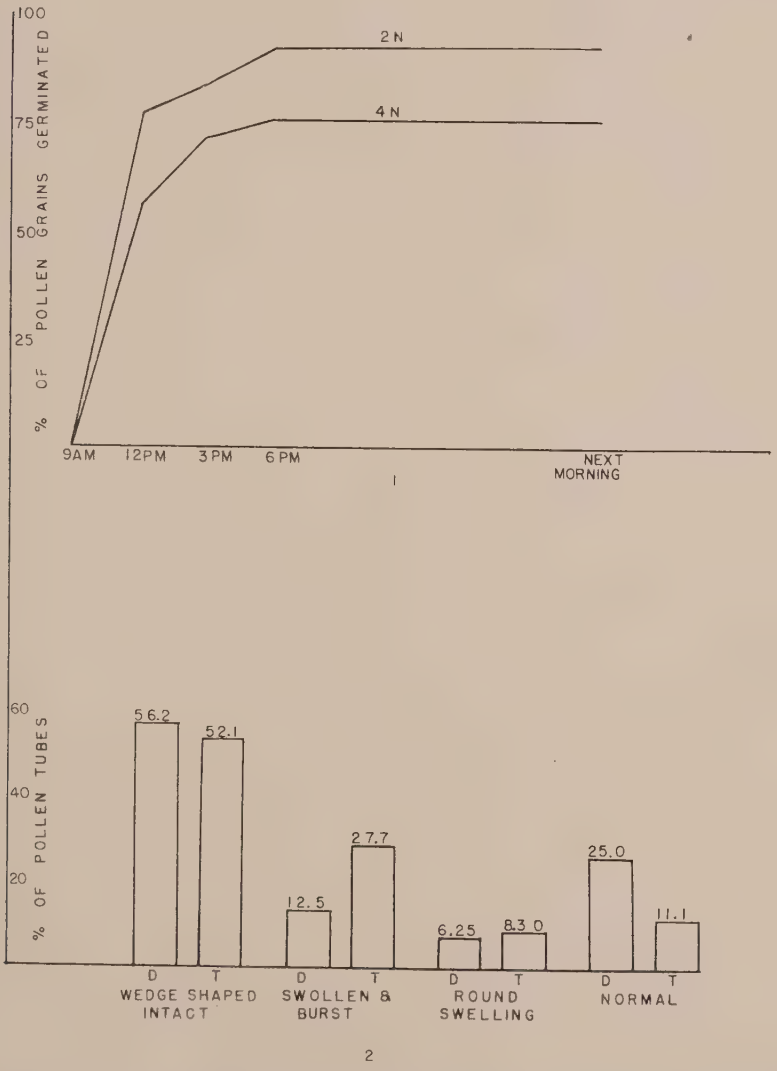

PLATE 4.—Pollen germination and pollen tube growth in diploid and tetraploid *Linum usitatissimum*. Fig. 1. Rate of pollen grain germination in diploid and tetraploid. Fig. 2. Relative percentages of different kinds of pollen tubes in diploid and tetraploid.

studied in more than 25 plants of the C_2 generation reliable chromosome counts could be obtained only in 17 plants. Table 5 gives the variation in chromosome numbers obtained in different tetraploid plants. Of the 17 plants studied only 9 had 60 chromosomes, while the remaining 8 had chromosome numbers ranging from 52 to 58. Thus 47.1 per cent of the observed plants were aneuploids and among them those with

TABLE 5.—*Frequency of tetraploid plants with different chromosome numbers.*

Type	No. of plants with chromosome numbers.									Total No. of plants
	52	53	54	55	56	57	58	59	60	
RR 63	1		1				1		4	7
RR 68					1		2		5	8
RR 39							2			2
										17

TABLE 6.—*Types of associations of chromosomes in three plants at metaphase I or late diakinesis.*

Plant No. 68–77–1=2 Chromosome No. 58					Frequency of cells	Plant No. 63–37–1=3 Chromosome No. 52					Frequency of cells	Plant No. 63–84–1=1 Chromosome No. 60					Frequency of cells
Types of associations						Types of association						Types of associations					
IV	III	II	II	I		IV	III	II	II	I		IV	III	II	II	I	
5	—	8	1	—	1	2	—	18	2	8	2	7	—	14	2	—	3
4	—	21	—	—	1	5	—	12	2	4	1	5	—	18	2	—	3
2	—	25	—	—	1	—	—	10	12	8	2	4	—	17	10	—	2
4	—	19	2	—	1	1	—	20	2	8	1	3	—	18	4	—	2
4	—	16	5	—	1	2	—	16	6	4	2	3	—	20	4	—	2
4	—	18	3	—	2	3	—	10	11	4	2	—	—	—	—	—	—
5	—	17	2	—	2	—	—	16	11	4	1						
5	—	16	3	—	3												
2	—	22	2	—	1												
6	—	14	3	—	1												
8	1	9	2	1	1												
4	—	17	4	—	2												
6	—	15	2	—	1												
2	—	21	4	—	1												
M.=4.4						M.=1.8						M.=4.4					

58 chromosomes were more frequent (62.5 per cent of the aneuploids). No plants with odd numbers of chromosomes were observed.

Meiosis:—The study of meiosis in tetraploids revealed many irregularities: (a) Metaphase I:—Plants with 60 chromosomes showed regular first metaphase plates but in some pollen mother cells a few bivalents or univalents were observed to lie at a distance from the equatorial plate. In 58-chromosome plants greater irregularities were observed than in the 60-chromosome plants. Aneuploids with less than

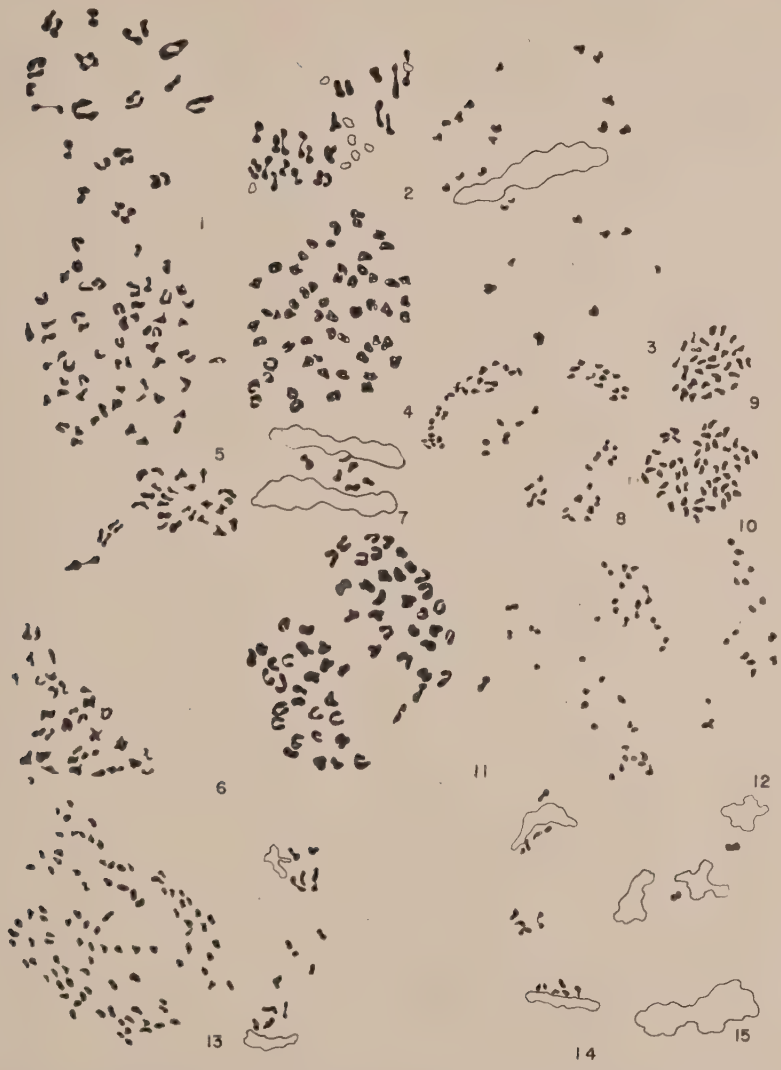

PLATE 5.—Meiotic and mitotic division in diploid and tetraploid *Linum usitatissimum*. Figs. 1–3. Tetraploid meiotic division Metaphase I. Fig. 1. 23 bivalents and 3 quadrivalents. Fig. 2. With 8 univalents. Fig. 3. Univalents scattered on both sides of the metaphase plate. Figs. 4–8 and 11–13. Tetraploid Anaphase I. Fig. 4. Early anaphase, 58 chromosomes. Fig. 5. Early anaphase showing splitting of chromosomes. Fig. 6. Late anaphase showing two lagging chromosomes divided into component chromatids. Fig. 7. Anaphase showing laggards. Fig. 8. Showing irregular distribution of chromosomes at anaphase. Fig. 11. Early anaphase showing unequal distribution of chromosomes. Fig. 12. Late anaphase showing laggards and splitting of chromosomes. Fig. 13. Early anaphase with some chromosomes split. Fig. 14. Late anaphase II, showing laggards. Fig. 15. Late telophase I with unequal distribution of chromatin content and two unintegrated chromosomes. Figs. 9 & 10. Mitotic metaphase plates of diploid (2n=30) and tetraploid (4n=58) respectively.

58 chromosomes showed large numbers of univalents and bivalents scattered away from the main plate. In the 52-chromosome plant many univalents were seen scattered throughout the cytoplasm on both sides of the equatorial plate.

Configurations comprising quadrivalents and bivalents were noticed in the different metaphase I plates studied. Trivalents were rare; one trivalent was found in a single cell. The quadrivalents observed were mostly of the ring type but occasionally chain type was also found. Most of the bivalents were of the ring type but rod bivalents were also common. Table 6 shows the frequency of the different types of associations of chromosomes observed in 3 tetraploid plants at late diakenesis or early metaphase I.

The number of quadrivalents varied from cell to cell. Plants with 60 or 58 chromosomes had a greater number of quadrivalents than did those with 56 or 52. Generally the number of quadrivalents varied from 4–8 per cell. Aneuploids with lower numbers of chromosomes had an increased frequency of univalents which were scattered on both sides of the metaphase plate (Plate 5, Figs. 1, 2 & 3).

(b) Anaphase I:—Plants with 60 chromosomes generally showed regular anaphasic separation. Rarely laggards were observed. Plants with aneuploid chromosome numbers showed laggards in varying numbers. In aneuploids with lesser numbers of chromosomes, abnormalities comprising lagging of univalents, and occasionally of bivalents, were found to occur in increased frequencies. At late anaphase, most of the chromosomes were grouped together at the poles (Plate 5, Figs. 6, 7, 8, 12 & 13).

Occasionally in some cells, at early first anaphase the univalent chromosomes were observed to divide equationally (Plate 5, Figs. 4, 5 & 13). In some cells at early and late anaphase as many as 80 to 94 distinct bodies could be counted, due to the early division of many of the chromosomes. In a cell at anaphase, 33 and 23 chromosomes were seen at the two poles together with one bivalent and two pairs of half chromosomes lagging between them (Plate 5, Fig. 6).

All these irregularities led to unequal distribution of chromosomes at the poles; thus 28 and 30, 23 and 31, and 26 and 30 chromosomes were counted in a number of cells at first anaphase (Plate 5, Fig. 11).

(c) Telophase I:—The chromosomes at the two poles formed daughter nuclei. At telophase, instead of two distinct groups of chromosomes sometimes 3 or more such congregations occurred. In many cases a few chromosomes were found close to the telophase nuclei without fusing with them. These were usually laggards which arrived later (Plate 5, Fig. 15). Such chromosomes either divided and took part in the second anaphase, usually forming laggards, or formed separate groups called micronuclei.

(d) Second division:—At second metaphase sometimes the two plates were quite near each other. The split univalent chromosomes were clearly observed even at early metaphase II and in one such cell a univalent laggard lying between the two metaphase II plates was seen completely divided into two halves.

Anaphasic separation was generally more regular at the second division than at the first but here also a few extreme cases of lagging

chromosomes were observed (Plate 5, Fig. 14). Generally laggards varied in number from one to four. In some cases, it was found that the second division was not simultaneous in both nuclei of the same pollen mother cell.

TABLE 7.—*Relationship between chromosome number and number of fruits and seeds.*

Plant and culture number	Total Number of Capsules	Average size of capsules in cm.	Average No. of seeds per capsule	Average size of seeds in mm.	Chromosome No. (4n & 2n)
Tetraploid					
RR 68					
62–1=1..	466	0.99 × 0.86	1.56	5.0 × 2.7	60
80–1=3..	351	1.03 × 0.90	3.60	5.3 × 2.8	60
62–1=2..	96	1.05 × 0.92	1.56	5.1 × 2.8	60
37–1=2..	261	1.01 × 0.88	3.20	5.2 × 2.8	60
77–1=2..	32	1.07 × 0.87	0.88	5.2 × 2.9	58
37–1=4..	37	0.94 × 0.83	0.84	5.0 × 2.9	58
37–1=1..	4	Varying small sizes	0		56
RR 63					
84–1=1..	460	1.00 × 0.87	2.80	5.2 × 2.7	60
76–3=6..	114	0.87 × 0.78	2.20	4.7 × 2.6	60
71–1=2..	260	0.95 × 0.80	3.00	5.0 × 2.6	60
63–4.....	40	0.94 × 0.82	0.82	5.0 × 2.7	58
63–1.....	6	0.91 × 0.77	0		54
37–1=3..	5	0.91 × 0.77			52
RR 39					
39–1.....	30	0.93 × 0.82	0.83	5.0 × 2.6	58
39–5.....	40	0.94 × 0.83	0.83	5.0 × 2.7	58
Diploid					
RR 68.....	480	0.89 × 0.83	9.80	5.1 × 2.5	30
RR 63.....	510	0.80 × 0.75	8.88	5.1 × 2.5	30
RR 39.....	445	0.85 × 0.76	9.80	5.0 × 2.5	30

TABLE 8.—*Frequency of tetraploid plants showing different average number of seeds per capsule.*

Type	Number of plants with the range of no. of seeds per capsule					Total No. of plants.
	0–1	1–3	3–4	4–5	More than 5	
RR 63...	3	10	16	13	1	43
RR 68...	2	7	2	0	0	11

At second telophase in many cases not all chromosomes were included in these nuclei. At the tetrad stage, 2–8 nuclei per cell were observed in a number of pollen mother cells.

(e) Cell formation:—After the two nuclear divisions were over the daughter nuclei took up nearly marginal positions in the cell. Furrowing of the cytoplasm followed and as many cells as there were nuclei, irrespective of their size, were formed. Diads, triads, pentads, hexads, octads, decads, etc. were observed in large numbers (Plate 3, Figs. 7,

8 & 9). These cells developed into pollen grains of varying sizes. In diploids, pollen grains contained 3 germ pores, whereas in tetraploids they generally had 4 (Plate 3, Figs. 5 & 6).

Mitotic Studies:—A number of miotic counts from microtome sections of root tips of diploid plants showed $2n = 30$ chromosomes. In spite of several attempts clear mitotic counts in root-tips of tetraploids could not be obtained due to precipitations in the cytoplasm. However, a few countable plates were obtained in one plant which revealed $2n = 58$ chromosomes; the number was in conformity with the meiotic number in this plant (Plate 5, Figs. 9 & 10).

8. *Seed sterility and fruit setting.*—(i) Relation with chromosome number of the plant:—Table 7 shows the total number of capsules, average size of capsules, average size of seeds, and average number of seeds per capsule in plants with different numbers of chromosomes. It is evident from the table that production of fruit and seed mostly took place only in plants with 60 chromosomes. With the exception of plants with 58 chromosomes, no aneuploid set any developed fruit or seed. The 58-chromosome plants bore some fruits but seed setting in them was very low in comparison to the normal 60-chromosome plants.

Thus, in aneuploids, in which meiosis was very irregular, the development of fruit and seed was seriously affected. Plants with 58 chromosomes showed less irregularity and consequently produced a few fruits and seeds.

Among the 60-chromosome plants there was considerable variation regarding the total number of capsules, the average number of seeds per capsule, and the sizes of capsules and seeds per plant. The variation ranged from 96 to 466 in respect of total number of capsules and from 1.56 to 5.1 in the average number of seeds per capsule. This large variation cannot be explained on the basis of the few cytological abnormalities observed at meiosis in microsporogenesis.

(ii) Variation in the C_2 population:—Table 8 shows that there was considerable variation with regard to seed setting in the C_2 population of tetraploid plants. The two types, RR 63 and RR 68, seemed to differ in this respect. The fact that it was possible to get one plant which set on average more than 5 seeds per capsule, indicated the possibility of selection for improved seed setting.

DISCUSSION

1. *Morphological characters.*—Morphological observations on diploid and induced autotetraploid *Linum usitatissimum* showed that tetraploids were bigger in size in almost all parts of the plant. This was in accordance with results obtained in most autotetraploid plants (Müntzing, 1936; Dermen, 1940).

There was a significant increase in tetraploids in the size of the seed—a character of great economic importance. Most of the seeds were, however, unfilled and shrivelled in appearance. The calculated 1000-seed weight in most of the plants was lower and only in a few plants was it equal to or slightly more than that of diploids. This does not support the report of Schlosser (1944) who stated that 1000-seed weight in polyploid linseed was higher than in diploid. It is not

known whether Schlosser's observations on 1000-seed weight were recorded on selected, fully developed seeds. In the present investigation all seeds set on a plant except the very shrivelled ones were included for recording 1000-seed weight.

A compensatory tendency in the development of flower was observed in one of the tetraploid strains of linseed; thus with increase in the size of anthers in RR 63 the size of filaments decreased. Such a tendency has also been reported in other parts of tetraploid plants such as leaves and fruits (Varaama, 1947).

During anthesis in some tetraploid plants of linseed the anthers, instead of normally holding the stigma from all sides, generally stretched away from the style and stigma, thus making natural self-pollination difficult.

The fiber bundles of the stem in tetraploids were narrower, smaller, loose, and distinctly separated from each other, whereas in diploids they were compact and formed a more or less continuous band. This would have an adverse effect on the quality of the fiber in tetraploids. Ross and Boyce (1946) also reported such loose structures of fiber bundles in tetraploid flax.

2. *Breeding behavior.*—The maintenance of polyploidy depends mainly on two factors:—(i) Regularity in cytological behavior. (ii) Incrossability with diploid progenitors or other allied species.

(i) Cytology:—Randolph (1942) stated that "Autotetraploids once produced tend to breed true for the doubled condition of their chromosomes except for deviation in number, of one or a few chromosomes that apparently do not affect the maintenance of the tetraploid state in succeeding generations." This statement, however, was not strictly applicable to the present tetraploid and to those of several other crops (Kadam, 1944; Ramanujam & Deshmukh, 1945).

In linseed, the progeny of tetraploids contained aneuploids to the extent of about 47 per cent. The aneuploids tended to be more variable in height than the euploid plants and could not be distinguished on the basis of general appearance, vigor or height but invariably through their extreme sterility.

The irregular distribution of chromosomes in tetraploids was largely attributed to the formation of multivalents at meiosis in them (Darlington, 1932). The number of quadrivalents in such plants may vary from zero to the maximum number expected depending upon the length of chromosomes (Kostoff, 1940), frequency of chiasma formation among the four homologues, the rate of terminalization of chiasma (Darlington, 1931) and the physiological condition determined in part by the environment (Müntzing, 1936). The frequency of chiasma formation and the rate of terminalization are not only mechanical properties of chromosomes but are also controlled by the genetic constitution of the organism (Randolph, 1941). Kadam (1944) attributed the difference in the frequency of quadrivalents in maize to the differences in their extent of heterozygosity.

The multiple associations of chromosomes result in the production of laggard bivalents or univalents at first anaphase, the lagging of whole or half chromosomes at second anaphase, non-inclusion of some chromosomes in the first and the second telophasic nuclei, and forma-

tion of pollen mother cells with a large number of micro-nuclei. A great range of functional pollen grains containing unbalanced numbers of chromosomes has been found in a number of autotetraploids. Besides these irregularities of meiosis, a number of cases of splitting of all or of a few chromosomes into their component chromatids between the stages of early anaphase I and early metaphase II have also been observed in two of the studied tetraploid plants of linseed. This type of abnormality has been described by Levan (1939) while studying the effects of colchicine on meiosis.

(ii) Crossability:—(a) Tetraploids selfed:—In linseed, seed setting after hand pollination seemed to be better than following natural selfing (bagged) or open pollination (no bagging). This suggested that natural selfing and open pollination might not provide enough good pollen per stigma to effect fertilization of all ovules. This point will be explained further when other factors causing seed sterility have been considered.

(b) Crosses among tetraploid strains:—In artificial pollinations, tetraploids readily crossed among themselves. However, in the reciprocal crosses between tetraploids of RR 63 and RR 68, it was found that when the latter was used as a female parent the number of capsules formed and the total number of seeds obtained were considerably more than when the cross was made in the other direction. As both RR 63 and RR 68 are equally poor in seed setting after selfing, the very high seed setting in the cross when RR 68 is the female parent and extremely low seed setting in the reciprocal cross when RR 63 is the female parent show that the difference in seed setting is not due to undeveloped embryo-sacs but may be due to defective pollen tube growth and post fertilization disturbances. This indicates the importance of the genetical constitution of the male and female parents in the development of seeds and capsules.

(c) Crosses among tetraploid and diploid:—Among the 47 crosses between 4n and 2n, partial development of as many as 23 capsules took place but only 3 seeds were produced. These 3 seeds produced normal diploid plants. In the reciprocal cross, $2n \times 4n$, a few shrunken capsules with no seeds were obtained. In the tetraploid population no triploids or near triploids could be identified, thus showing that the diploid and the tetraploid forms of linseed did not intercross under natural conditions. Levan (1948), on the other hand stated that "while diploid flax is considered a rather strict self-fertilized, the tetraploids readily cross with diploids." This indicated an important difference between tetraploids of flax (fiber) and linseed (oil). The high percentage of incrossability between diploids and tetraploids is a very valuable economic character in tetraploid linseed as it would help in maintaining the purity of the tetraploid populations.

3. *Sterility.*—Autotetraploids are often characterized by reduced fertility of pollen and seed. However, some workers have reported high fertility in induced autotetraploids (Langham, 1942; Nilsson, 1948).

Demerec (1947) states that there are at least 3 different causes of sterility in autotetraploids:—(i) irregular chromosome distribution caused by unequal distribution of multivalents (Darlington, 1937; Kostoff, 1940). (ii) Irregular distribution of chromosomes caused by

meiotic abnormalities of a physiological nature, presumably controlled genetically (Müntzing, 1936; Randolph, 1941) and (iii) Genetic-physiological sterility of an unexplained nature but not associated with meiotic irregularity (Randolph, 1941; Kadam, 1944). That sterility was not always due largely to multiple association of chromosomes has been shown by high fertility in some natural and artificial autotetraploids having a high percentage of multivalents (Müntzing, 1936; Myers, 1943).

Thus sterility in autotetraploids appears to be of a complex character. The high percentage of pollen fertility (Ramanujam & Deshmukh, 1945) and low production of seeds indicate that sterility may be exhibited at any stage of the various processes leading to full development of seed, viz. the germination of apparently fertile pollen grains, normal growth of pollen tubes, full development and maturity of embryo-sacs at the time of pollination and subsequent normal development of the zygote or embryo.

Moreover it is very likely that more than one factor may be responsible for sterility in any particular crop. Einset (1944) attributed 3 causes for sterility in tetraploids of *Lactuca sativa:*—(i) Irregular distribution of chromosomes at meiosis in pollen mother cells, (ii) Complete breakdown of meiosis in the 20 per cent of megaspore mother cells and (iii) partial incompatibility in pollen tube growth. In the case of linseed, the following factors seem to be jointly responsible for sterility in tetraploids:—(i) Meiotic abnormalities in pollen mother cells are common. (ii) failure of the majority of embryo-sac (70 per cent) to attain the fully matured, 8-nucleate stage at the time of fertilization. Although it had not been possible to record observations on meiosis in megaspore mother cells in the present material, observations indicated that unlike the observations of Einset, meiosis in megaspore mother cells in linseed generally appeared to have taken place but the division of megaspores could not proceed further than the 2- to 3-nucleate stages of the embryo sac. The structure of the nuclei indicated that there was some serious upset in the organization of the chromatin in them. (iii) In some tetraploid plants there was considerable sterility presumably as a result of the defective mode of anther dehiscence which made self-pollination difficult. (iv) Non-germinability of 13.8 per cent of the apparently fertile pollen grains. (v) Of the total number of pollen tubes only a bare 11.1 per cent were of the normal type (diploid—25 per cent), as against 88.9 per cent of abnormal types of pollen tubes many of which probably burst or aborted during their way down the style. According to these observations only 8–9 per cent of all pollen grains per stigma would be capable of effecting fertilization. The better seed setting in tetraploid linseed when hand pollinated as compared to natural selfing or open pollination may therefore be due to the abundance of pollen in artificial pollination. Thus there is considerable evidence that abnormalities both on the male and female sides, were jointly responsible for seed sterility in tetraploid linseed.

4. *Partial self-incompatibility.*—In autotetraploid *Lactuca sativa*, Einset (1944) observed that the failure of the pollen to germinate and grow in the styles of the mother plant was the chief cause of sterility. Diploid *L. sativa* produces full seed set. In the present study of *L.*

usitatissimum it was found that the percentage of pollen germination per stigma was lower and percentage of abnormal pollen tubes higher in tetraploids than in diploids. The cyclic mid-season fertility commonly observed in certain self-incompatible plants of *Brassica pekinensis* and *B. chinensis* was also found in autotetraploid *L. sativa*. These observations indicate an expression of partial self-incompatibility in autotetraploids which was not detected in diploids. In *Dentaria bulbifera* whose meiotic behavior suggested that it was an autotetraploid, pollen tubes failed to reach the bottom of the style (Schwarzenbach, 1922).

Species with the gametophytic oppositional incompatibility system, in which autotetraploids have been examined, show that with one possible exception (*Oenothera rhombipetala*—Hecht, 1944) autopolyploidy brings about self-fertility (Lewis, 1954) in all of them. In self-incompatible heterostyled species with sporophytic control of pollen and gametophytic control of style, only rare self-fertile homostyle plants have been observed among their autotetraploids. But in *Parthenium argentatum* (*Compositae*) and *Brassica* species (*Cruciferae*) in which self-incompatibility is sporophytic in both pollen and style, autotetraploids remain fully self-incompatible. In *Raphanus sativus* (*Cruciferae*) in which self-incompatibility has not been worked out but in which sporophytic system is strongly suggested (Lewis, 1954), polyploidy does not produce self-fertility.

Thus it is significant that breakdown of self-incompatibility in diploid pollen is wide-spread only in the gametophytic system of incompatibility where no selection was possible against gene interaction in pollen. In diploid pollen with two unselected genes, competition between alleles was possible giving self-fertility. In the tetraploid forms of heterostyle plants pollen is under sporophytic control and is therefore selected for gene action; here no breakdown occurred. The only breakdown in the system was probably due to an increase in crossing over within the S supergene resulting from the pairing properties of a quadrivalent—a situation which would not have been subjected to selection (Lewis, 1954). In species with sporophytic control of pollen and style reaction two alleles are normally present at the time of gene action and thus there has been strong selection for the type of allele interaction. Therefore, negative effect of polyploidy in the sporophytic system is to be expected.

Lactuca sativa and *Linum usitatissimum* belong to the families *Compositae* and *Linaceae*, respectively, in which certain other species are self-incompatible and have been known to belong to the sporophytic system of self-incompatibility. *L. sativa* and *L. usitatissimum* give full seed set in the diploid and are therefore considered fully self-fertile species. But pollen tube studies in diploid *L. usitatissiumum* showed inhibition in pollen germination and considerable abnormalities in pollen tube growth. Thompson (1933) reported a certain amount of failure of fertilization in diploids in *Brassica oleracea* (*Cruciferae*) as did Ekdahl (1949) in *Galeopsis pubescens* (Labiatae). *Cruciferae* have a sporophytic system of self-incompatibility. The system of self-incompatibility in *Labiatae* is not known but the above mentioned behavior may suggest that it also has a sporophytic system.

Considerable evidence indicates that most flowering plants have at

present or have had in the past a self-incompatibility system, and that the species which are now self-compatible have been derived from a self-incompatible ancestor. The *Cruciferae* have about half its species self-incompatible and about half self-compatible. Some genera appear to have species that are exclusively of one type: this indicates that self-incompatibility or self-compatibility, as the case may be, has been established in these species for a long time (Lewis, 1955). But other genera, as *Linum* in *Linaceae*, have both types of species suggesting that self-compatibility is of recent origin.

Once an organism has no further use for a process or structure it tends to lose it gradually, sometimes never losing the last vestige. In *Trifolium pratense* (Pandey, 1955a) which is a self-incompatible species, mechanical barrier to fertilization which probably was helpful in the early evolution of self-incompatibility, has become a stable phenomenon though at the present it has no connection with the incompatibility system. In diploid *L. usitatissimum* abnormal pollen germination and pollen tube growth indicate that this species has recently become self-fertile and though the species is fully seed fertile, self-incompatibility has not been fully removed. In species like *L. usitatissimum* and *L. sativa* in which the number of ovules to be fertilized in the ovary is relatively small, partial self-fertility gives enough normal pollen tubes to produce full seed set. Partial self-fertility in such species can easily be a stable phenomenon as the selection for full fertility could not operate efficiently beyond that stage. Partial self-incompatibility can only be detected in species which have a large number of ovules to be fertilized in an ovary. Thus the species which produce only a few seeds per ovary can be found partially self-fertile as a stable state but species with a large number of ovules to be fertilized in an ovary will tend to be fully self-fertile by selection.

In the light of the present discussion, it is suggested that the two species, *Linum usitatissimum* and *Lactuca sativa*, in which diploids are fully seed-fertile and autotetraploidy has produced a certain degree of self-sterility, were recently self-incompatible and, like certain related species, belonged to the sporophytic system of self-incompatibility. In autopolyploids with a sporophytic system new dosage relationships are possible and, because weak and complete dominance are features of this system, it is possible that such new dosage relationships may actually increase self-incompatibility rather than decrease it as is found in a gametophytic system.

If the above statement is generally true, it may be concluded that in families with a sporophytic system of self-incompatibility autopolyploidy in self-fertile species of a genus which has also self-incompatible species, would lead to more seed sterility than would commonly be expected from their cytological behavior. No wonder, autopolyploidy has failed to produce higher (seed) yielding varieties of crop plants in self-fertile or partially self-fertile species of *Cruciferae*, *Compositae*, and *Linaceae*, all three possessing sporophytic systems of self-incompatibility.

5. *Conclusions.*—The various observations on morphological and cyto-genetical characters of the three strains of induced tetraploids of linseed indicated that there was a great variation regarding these

characters among the strains. There is a wide range of fertility varying from 0–6 seeds per capsule in tetraploids as compared to 8–10 seeds in diploids. Different plants of a strain differ regarding their capacity to give out fertile progeny. Even the progeny of the same plant show varying fertility. The fact that at least a few plants with as high fertility as 5–6 seeds per capsule could be obtained in the C_2 generation indicates that by treating a large number of genetically varying materials with colchicine and thus increasing the scope of selection, it may be possible to get a few plants of linseed with very high fertility which may still be improved by further breeding of the promising plants.

SUMMARY

Induced autotetraploids of three strains of linseed, *Linum usitatissimum* L., were studied to observe their comparative morphological and cytogenetical behavior in the C_2 population, with special reference to the factors causing seed sterility.

A study of transverse sections of stems revealed considerable difference between the fiber bundles of diploid and tetraploid strains the latter being commercially inferior to diploid.

There was a conspicuous difference in the reciprocal crosses of tetraploids presumably due to the incompatible genetical constitution of the female parent.

Crosses between 2n and 4n plants showed that they were highly incompatible—an economic character of great significance for it ensures the purity of the tetraploid strains.

Cytological studies showed that about 47 per cent of the tetraploid plants were aneuploids. Meiotic studies revealed various irregularities at different stages of first and second divisions. Of the aneuploids, only 58-chromosome plants produced a few seeds.

The following factors seemed to be jointly responsible for sterility in tetraploid linseed:—(1) Meiotic abnormalities in pollen mother cells were common. (2) The majority of embryo-sacs (70 per cent) failed to attain a fully mature, 8-nucleate stage at the time of fertilization. (3) In some tetraploid plants (about 10 per cent) there was considerable sterility presumably as a result of the defective mode of anther dehiscence which made self-pollination difficult. (4) Of the apparently fertile pollen grains 13.8 per cent failed to germinate. (5) Of the total number of pollen tubes only 11.1 per cent were of the normal type (diploid—25 per cent), compared with 88.9 per cent of abnormal types of pollen tubes, many of which burst or aborted during their way down the style. Thus only 8–9 per cent of the pollen grains on the stigma were fully capable of effecting fertilization.

The failure of some pollen grains to germinate and considerable abnormality in pollen tube growth indicated that partial self-incompatibility may have been in operation. A hypothesis explaining the occurrance of such partial self-incompatibility in autotetraploids was proposed.

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Floral Morphology and Embryology of Balanites roxburghii Planch.

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The systematic position of the genus *Balanites* is doubtful. It has been treated under Simarubaceae by Bentham and Hooker (1862) and under Zygophyllaceae by Engler and Prantl (1931). Record (1921) stated that the wood structure of *Balanites* has no resemblance to that of other members of the Zygophyllaceae. Heimsch (1942) considers that apart from the rays the wood anatomy of *Balanites* suggests affinity with the Zygophyllaceae rather than with the Simarubaceae. Elsewhere, however, he states that "The high and wide rays of *Balanites* may be sufficient to exclude the genus from Zygophyllaceae." In view of these conflicting opinions and since there is no previous work on the various aspects of floral morphology of the genus the present problem was undertaken.

MATERIAL AND METHODS

The material of *Balanites roxburghii* was collected in Pilani from April, 1953 to May, 1955. Formalin-acetic-alcohol was used as the fixative. Dehydration and embedding were followed according to the ethyl alcohol xylol series and sections were cut at 6–14 μ thick. They were stained with safranin, fast green, and iron-alum-haematoxylin, the latter giving better result for embryological studies. For floral anatomy staining with erythrosin and crystal violet was also carried out.

OBSERVATIONS

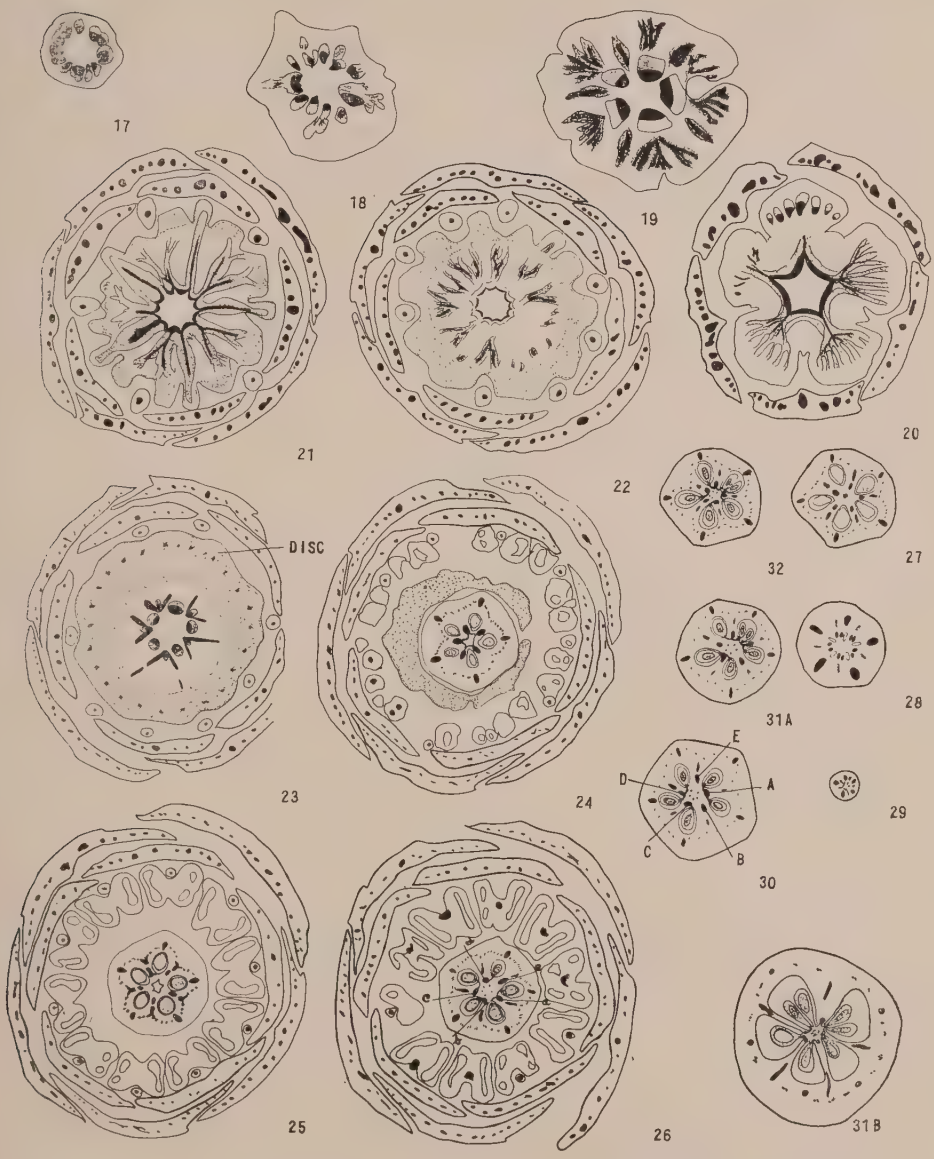
Morphology of the flower.—*Balanites roxburghii* is a thorny evergreen tree characteristic of the desert areas of Rajasthan. The flowers are borne on axillary three to nine flowered inflorescences. The small, green flower arises in the axil of a caducous scale like a bract, is pentamerous (sometimes hexamerous in all cycles), hypogynous, and dichlamydeous. A conspicuous intra-staminal disc envelops part of the ovary (Fig. 2). The style is short and the stigma is truncate. The ovary is five celled and each locule contains a single ovule but occassionally two ovules may be present (Figs. 31, 32). The outer surface of the sepals and the pedicel are covered with unicellular hairs (Fig. 4). Sphaerocrystals are present in all parts of the flower. Mucilage secreting cells are present in the margin of the disc. The floral parts arise in acropetal succession (Figs. 5–7).

Vascular anatomy of the inflorescence.—The stele in the lower region of the peduncle consists of a ring of ten to twelve endarch bundles of somewhat unequal size (Fig. 8). From the central stele diverges a trace to each of the caducous bracts arranged in a spiral (Figs. 9–11). This is followed by the vascular supply of the flowers subtended by the bracts (Fig. 10). After giving supply to the pedicel of the uppermost flower the resedual stele vanishes at the tip (Figs. 15, 16).

Vascular anatomy of the flower.—The pedicel contains a ring of twelve to fourteen bundles (Fig. 17). Each of the five traces of the first whorl, the commissural laterals of sepals, while moving to the periphery of the receptacular cortex, divides to form a number of branches (Figs. 18, 19). The next whorl of traces constitutes the sepal midribs (Fig. 19). Above this level the stele assumes a pentagonal shape and from the angles are given off the petal traces in radii alternating with the sepal midribs (Fig. 20). At the base of each petal the

FIGS. 1–15.—FIG. 1. A young three flowered inflorescence. x 1.25. FIG. 2. An opened flower. x 2.5. FIG. 3. L.s. of flower bud. x 13. FIG. 4. Unicellular hair from sepal. x 178. FIGS. 5–7. Organogeny. FIGS. 5, 6. x 113. FIG. 7. x 69. FIGS. 8–16. Successive transections of the flower from the pedicel upward. x 13.

petal trace divides to form five to seven branches. The gaps formed by the petal traces are bridged over and from the circular stele diverges the supply for the stamens. Of the two whorls of stamens, the outer antisepalous whorl receives the supply first (Fig. 21). Each staminal trace divides tangentially into two parts, the outer part entering the stamen while the inner ramifies the disc (Figs. 21, 22). The receptacular stele again assumes a pentagonal shape and from the angles are given off at the petal radii five dorsal traces of the carpels each of which, soon after its origin, divides into three (Fig. 23). The lateral branches ramify in the ovary wall. The dorsal traces are followed by the fused secondary marginals of adjacent carpels (Fig. 25) which also take part in the supply of the ovary wall.



FIGS. 17–32.—FIGS. 17–29. Successive transections of the flower from pedicel upwards showing the vascular supply. In FIGS. 27–29 only the gynoecium is shown. FIGS. 29–32. T.s. of ovaries showing variation in the supply to the ovules. x 13. *a*, *b*, *c*, *d* and *e* are the placental bundles.

The remaining vascular tissue forms a pentagonal structure and gives off five traces which become the placental bundles. They take their positions in septal radii (Fig. 25). The bundles are first normally oriented with respect to the central axis, and become inversely oriented in the higher region. After the differentiation of the supply to the carpels there remain in the center a few vascular elements, chiefly phloem, which fade out in a few sections (Figs. 25–28).

The supply to the ovules shows some variation. Of the five placental strands bundle *a* supplies two ovules and bundles *b*, *c*, and *d* supply one each, while bundle *e* does not supply any ovule (Fig. 26). In some cases bundle *d* also supplies two ovules in which case bundle *b* takes no part in ovular supply (Fig. 30). In still other cases where there are two ovules in some of the carpels they were supplied by placental strands on either side of the locule (Figs. 31*a*, *b*, 32).

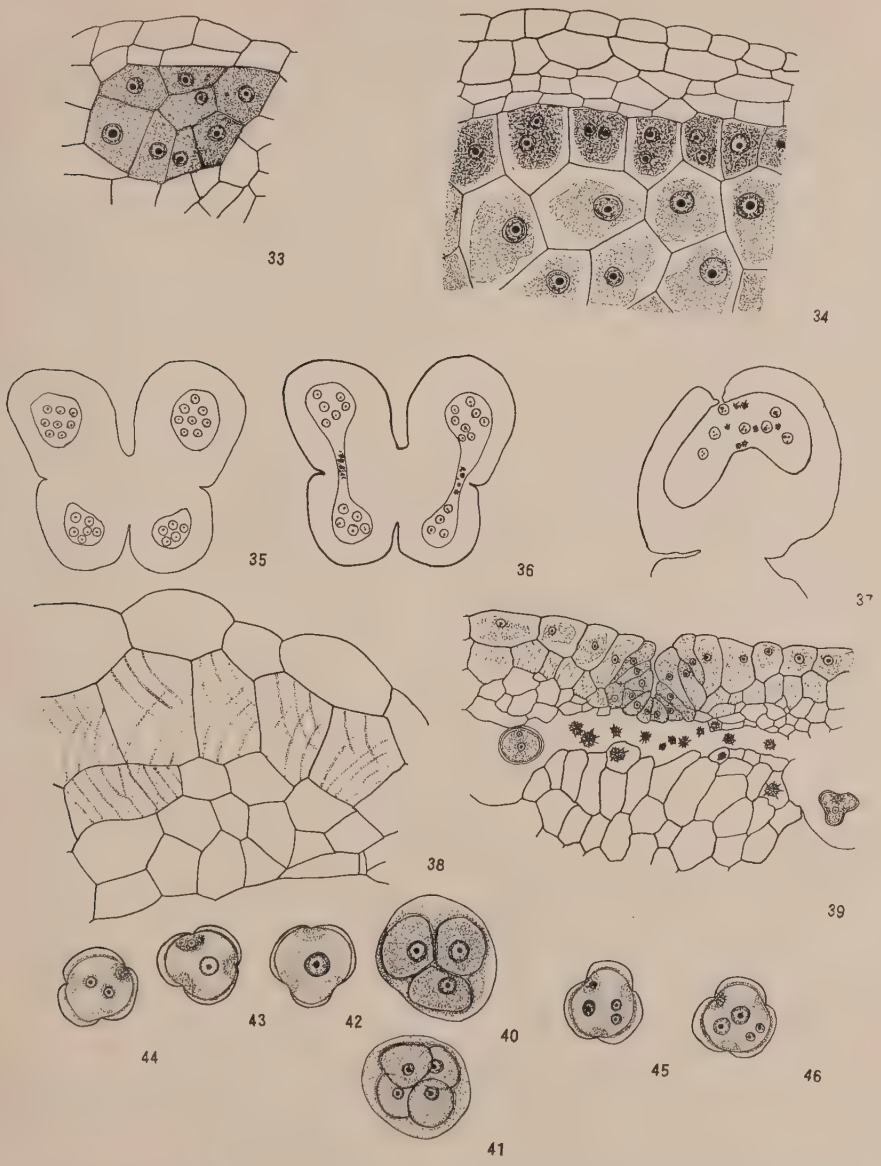
The placental bundles divide to form two bundles each at the base of the style (Fig. 28). These bundles plus the carpellary dorsals and secondary marginals constitute the supply of the style (Fig. 29). All of them fade out as the stigmatic region is reached.

Microsporogenesis and male gametophyte.—The young anther becomes four lobed and each lobe develops into an elongated sporangium. The cells of the partition wall between adjacent pollen sacs contain sphaerocrystals. At maturity when the two pollen sacs become confluent due to the breaking down of the partion wall between them the sphaerocrystals are liberated in the sporangial cavity (Figs. 35–37, 39). In a mature anther the pollen grains are seen mixed with sphaerocrystals.

In each lobe of the anther appears a plate of hypodermal archesporial cells which divide to form a primary parietal layer and sporogenous tissue (Fig. 33). The former, following another division, gives rise to an outer layer, the endothecium, and an inner layer which gives rise to the tapetum and the middle layers. The tapetal cells are uninucleate at first but become binucleate before the microspore mother cells enter reduction division (Fig. 34). The tapetum is of the secretory type. At the time of tetrad formation the tapetal cells also lose contact with each other. The cytoplasm becomes vacuolated even earlier and shows signs of degeneration. At the uninucleate stage of the pollen grain no remnant of the tapetum is seen. The middle layers persist even at maturity (Fig. 38, 39). Some of the cells of the middle layers simulate the endothecial cells by developing fibrous thickenings (Fig. 38).

The dehiscence of the anther lobes takes place along the line of partition between two microsporangia on each side of the anther where the cells are thin and narrow (Figs. 35, 36, 39). Each anther lobe has three to four layers of compactly arranged microspore mother cells. The microspore mother cells undergo simultaneous reduction division and cytokinesis takes place by furrowing. The tetrads are usually tetrahedral but decussate tetrads are not infrequent (Figs. 40, 41). The mucilaginous wall secreted by the microspore mother cells during reduction division becomes more prominent at the tetrad stage but is consumed as soon as the uninucleate microspores separate from one another.

The uninucleate microspore has a centrally placed prominent



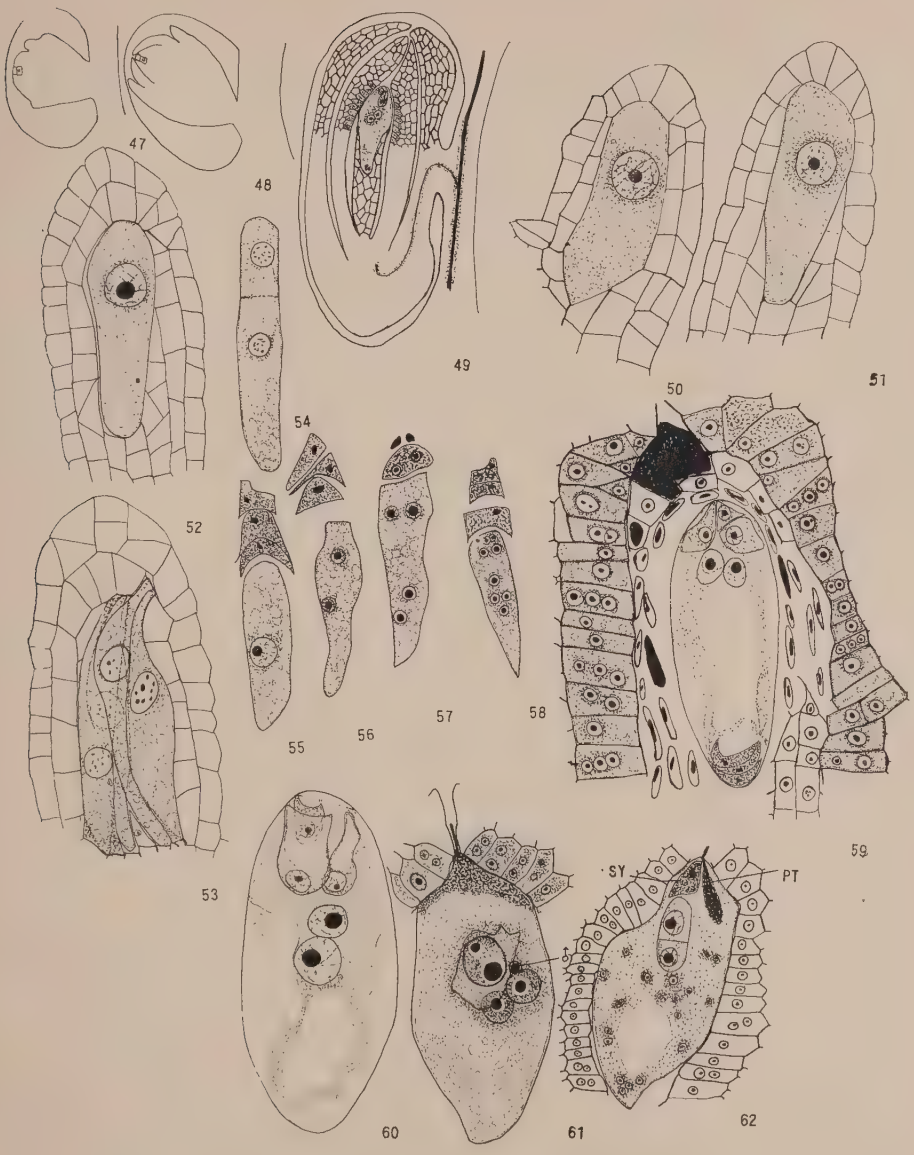
FIGS. 33–46.—FIG. 33. T.s. of young anther lobe showing hypodermal archesporium and primary parietal layer. x 313. FIG. 34. The same at microspore mother cell stage. x 313. FIGS. 35–37. Outlines of the anther lobes to show the dehiscence of the anther. x 38. FIG. 38. Part of anther lobe at the time of dehiscence to show the wall layers. x 313. FIG. 39. Part of anther lobe showing cellular details of the region of dehiscence. x 250. FIGS. 40, 41. Tetrahedral and decussate tetrads. x 313. FIG. 42. Uninucleate pollen grains. x 313. FIGS. 43, 44. Two celled and three celled pollen grains. x 313. FIGS. 45–46. Abnormal pollen grains showing supernumerary nuclei. x 313.

nucleus (Fig. 42). As the spore enlarges in size the nucleus moves to one side where it divides to produce a small generative cell and a large vegetative cell. The two cells are separated by a membrane but due to the early dissolution of this membrane the generative cell moves and comes to lie adjacent to the vegetative nucleus where it undergoes another division to produce two sperm cells which are spherical (Fig. 44).

The mature pollen grains are triporate and psilate. Pollen grains containing supernumerary nuclei have been frequently observed. Most of such grains have four nuclei of which two were large and looked like vegetative nuclei while others were small (Fig. 46). The additional nuclei might have been formed as a result of disturbances in microspore cytology. Pollen grains with supernumerary nuclei formed by the division of either the vegetative or the generative cell have been known in a considerable number of plants.

Megasporangium and megasporogenesis.—The ovules are crassinucellate and bitegmic. They arise as small protuberances and become campylotropous by the time the megaspore mother cell is distinguished. The inner integument and the archesporial cell make their appearence at about the same time. Both integuments are considerably enlarged by the time the reduction division is over. Rarely both integuments are fully developed even at the megaspore mother cell stage. The inner integument is 3–6-layered and the outer integument is 4–8-layered. The micropyle is narrow and formed by both integuments (Fig. 49). The exostome does not lie in a line with the endostome such that the micropyle shows a zig-zag outline. The vascular supply to the ovule terminates in the chalaza. The nucellus is poorly developed and separated from the inner integument by a conspicuous space in the chalazal region (Fig. 49). The inner and outer integuments are also separated by a space. Due to lateral enlargement of the embryo sac the nucellar cells are crushed so that the former comes to lie in contact with the inner epidermis of the inner integument which differentiates into an endothelium (Figs. 49, 59, 62). The cells of the endothelium contain one to three nuclei. Only in the chalazal part occur five to six layers of nucellar cells.

The archesporium is hypodermal. It consists of one to four cells (Figs. 50, 51). When it is multicellular, the cells lie either side by side or one above the other. Usually a single cell functions but rarely more cells may develop into megaspore mother cells (Fig. 53). The archesporial cells undergo periclinal divisions resulting in a primary parietal layer which by further division result in a parietal tissue of three to four layers. The megaspore mother cell gives rise to the usual dyad and tetrad stages (Figs. 54, 55). The upper dyad cell is smaller than the lower. The tetrads are linear (Fig. 55). Some times the upper dyad may divide by an oblique wall forming T-shaped tetrads. The chalazal megaspore enlarges. Its nucleus divides thrice successively to given rise to an eight nucleate Polygonum type of embryo sac (Figs. 55–60). The rest of the megaspores degenerate. Degeneration begins from the micropylar end. The remnants of the degenerated megaspores are some times seen even at the eight nucleate stage. Fig. 57 shows twin embryo sacs, the upper smaller being binucleate and the lower one four-nucleate.



FIGS. 47–62.—FIGS. 47–49. Stages in the development of ovule. x 63. FIGS. 50, 51. Hypodermal archesporial cells from two consecutive sections. x 313. FIG. 52. Primary parietal layer and megaspore mother cell. x 313. FIG. 53. A group of megaspore mother cells. x 313. FIG. 54. Megaspore mother cell after the first meiotic division. x 313. FIG. 55. Linear tetrad of megaspores, the upper three of which are degenerating. x 313. FIGS. 56–60. Stages in the development of the embryo sac. x 313. FIG. 61. Double fertilization and triple fusion. x 313. FIG. 62. Free-nuclear endosperm and two celled proembryo. *syd*, synergid; *pt*, pollen tube. x 313.

The synergids are hooked and show apical vacuoles and basal nuclei (Fig. 60). Thus they simulate the egg. The synergids degenerate shortly after fertilization. In a few cases one of the synergids was seen to persist until a two celled proembryo is differentiated (Fig. 62).

The polar nuclei meet in the center of the sac and lie side by side. They move up and come to lie close below the egg apparatus (Figs. 59–61). The chalazal polar nucleus is bigger than the other.

Fertilization and endosperm.—Fertilization is porogamous. The pollen tube is ephemeral but some times persists even at the two celled stage of the proembryo (Fig. 62). Fig. 61 shows the post fertilization stage of an embryo sac where the two polar nuclei remain in association and one sperm lying close by. It is presumed that all three nuclei fuse at the same time. Syngamy precedes triple fusion. The antipodals are crescent shaped (Fig. 59) and in an embryo sac ready for fertilization no trace of them can be seen (Fig. 60).

The primary endosperm nucleus divides soon after triple fusion. The early divisions are not followed by wall formation and therefore the endosperm is free nuclear. The zygote rests for some time and it divides when about twenty endosperm nuclei are formed. The first division is transverse (Fig. 62).

DISCUSSION

The inflorescence of *Balanites roxburghii* consists of three to nine flowers. Hooker (1875) states that the inflorescence is an axillary cyme. It has been pointed out earlier that the vascular supply to the flowers arises in acropetal succession and when the inflorescence reaches a stage beyond which it cannot develop the residual vascular elements which normally would have been used up by succeeding flowers vanish towards the tip. These features show that the inflorescence in *Balanites roxburghii* is a raceme.

Bonnier (1879) stated that there can be hardly any morphological classification of the disc in various families because of the great variation found even in members of the same species. However, in many families the disc is supposed to be of great significance since it represents vestigial organs. A survey of the literature—which is too abundant to be included here—shows that the disc in various families has been interpreted differently by, namely as stipules, leaf, perianth, receptacle, stamens, carpels, etc. The ten lobed disc of *Balanites roxburghii* derives its vascular supply from the staminal traces which suggests that the disc in this species may be staminal in nature.

In the taxonomic literature placentation in *Balanites* is described as axile. It has been pointed out that the placental bundles are in the septal radii and that they divide into two each at the base of the style. When two ovules are present per carpel, they derive their vascular supply from placental strands on either side of the locules. These features suggest the heterocarpous nature of the placentas. The placental bundles are inversely oriented in the higher regions. In a typical axile placentation the placental bundles are found opposite the dorsal bundle and are formed by the fusion of the ventrals of the same carpel (see Puri, 1952). In the present case their position and behavior clearly indicate that placentation in *Balanites* is anatomically parietal even though the ovary is multilocular.

Joshi (1947) stated that parietal placentation in all cases is derived from axile. Gundersen, (1939), on the other hand, believes that axile placentation is derived from parietal placentation. Puri (1945, 1947, 1950, 1954) has shown that in many families parietal placentation is derived from axile. He is of the opinion that the inverse orientation of the placental bundles in parietal placentation is a "relic of the past" axile placentation. In this light the parietal placentation of *Balanites roxburghii* is also derived from axile placentation.

Tendency toward the reduction in number of ovules per carpel seems to be a feature of *Balanites roxburghii*. The number of ovules per locule may be either one or two. Whenever two ovules are present, they derive their supply from two placental strands on either side of the locule. In this case both margins of the carpel are fertile. In the carpels with single ovules, only one of the margins has retained fertility. Each placental bundle may supply either two ovules—one on either side of the placenta—or a single ovule or none. Thus it may be assumed that the immediate ancestors of *Balanites* had both margins of the carpel fertile, a condition that is still exhibited in some cases, and that stability has not been attained in present day forms.

It has been pointed out that after the placental strands have diverged there remain a few residual vascular elements which fade out in a few sections. This feature bears out the fact that the floral parts are lateral organs on a central axis.

The following table gives a comparison of the embryological features of *Balanites*, *Zygophyllaceae*, and *Simarubaceae* (for details see Schürhoff, 1924, 1926; Wiger, 1935; Rau, 1941).

Characters	*Balanites*	Zygophyllaceae	Simarubaceae
Middle layers of anther wall	Persistent	Ephemeral	Ephemeral
Pollen grains	Three celled	Three nucleate	Two celled
Ovule	Bitegmic crassinucellate and campylotropous. Micropyle formed by both integuments.	Bitegmic crassinucellate and anatropous. Micropyle formed by inner integument.	Bitegmic crassinucellate and anatropous. Micropyle formed by inner integument.
Nucellus	Very thin and ephemeral. Only a few cells persist in the chalaza.	Massive	Massive
Space between integuments	Present	Absent	Absent
Space between integument and nucellus	Present	Absent	Absent
Hypostase	Absent	Present	Present
Endothelium	Present and conspicuous	Absent	Absent
Size of polar nuclei	Unequal	Equal	Equal
Antipodals	Crescent shaped	Triangular	Triangular

As will be seen from the above, *Balanites* has several significant embryological features characteristic of it and different from those of Simarubaceae and Zygophyllaceae. This suggests the need of a

reexamination of the systematic position of *Balanites*. Further comparative work on representative genera included in different families of the order Geraniales as well as on other incompletely known genera of Zygophyllaceae and Simaroubaceae is necessary before the correct systematic position of the plant can be determined. For the present it seems that *Balanites* has no close connection with either Simarubaceae or Zygophyllaceae.

SUMMARY

Flowers are pentamerous throughout. The floral parts arise in acropetal succession. The disc receives its vascular supply from the stamens and therefore is regarded as staminal in nature. Placentation is considered to be parietal. After the supply of the various floral organs there remain a few residual vascular elements, which may be regarded as evidence that floral organs are lateral appendages on a central axis.

The anther wall consists of five layers. Tapetum is secretory and multinucleate. The endothecium is fibrous. Middle layers persist in the mature anther. Pollen tetrads are either tetrahedral or decussate. Pollen grains are shed at the three celled stage.

Ovules are bitegmic, crassinucellate, and campylotropous. The micropyle is narrow and formed by both integuments. The nucellus and the inner integument, and, the integuments are separated by spaces. The archesporium consists of 1–4 cells. The development of the embryo sac conforms to the Polygonum type. The synergids show apical vacuoles and basal nuclei. The polar nuclei are dissimilar. The antipodals are crescent shaped and ephemeral. Due to the lateral growth of the embryosac the nucellus is crushed and the former comes in contact with the inner integument which develops into an endothelium.

Syngamy precedes triple fusion. The endosperm is free nuclear. The zygote divides by a transverse division when about twenty endosperm nuclei are formed. The systematic position of *Balanites* is briefly considered in the light of embryological features.

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New genera, species, etc., are indicated by *italics*.